Annals
of the
Missouri Botanical Garden

VOL. I MAY, 1914 No. 2

A CONTRIBUTION TO OUR KNOWLEDGE OF THE RELATION OF CERTAIN SPECIES OF GRASS-GREEN ALGÆ TO ELEMENTARY NITROGEN

JACOB R. SCHRAMM

Assistant to the Director of the Missouri Botanical Garden
Instructor in the Henry Shaw School of Botany of
Washington University

A general survey of the literature pertaining to the relation of algæ to free atmospheric nitrogen reveals the fact that comparatively few forms have been experimented with under conditions which render the conclusions reached free from objection. The principal fault which may be found with most of the work done is that the experiments were carried out with impure cultures. Representatives from not more than four or five genera of green algæ have thus far been studied in pure culture, and while the general conclusion reached is that these forms are unable to fix free atmospheric nitrogen either in the presence or in the absence of combined nitrogen and energy-furnishing materials, it is by no means certain that forms do not exist which, under one or all of these conditions, are able to utilize elementary nitrogen. This thought is especially justified when the small number of free-nitrogen-fixing species among the bacteria is considered. In the present investigation, therefore, an attempt has been made to extend the observations over a greater variety of forms in pure culture,—understanding by the latter a single species of alga free from all other organisms.

Historical

As early as 1854 Laurent (20, 21), and Morren (24) occupied themselves indirectly with the relation of algæ to free atmospheric nitrogen. Morren was led to the conclusion that the sudden death of cultures of infusoria and algæ was due to the insufficient quantity of combined nitrogen furnished when the number of organisms became considerable. The nitrogen requirement, he found, could be satisfied by ammonium carbonate, organic nitrogenous compounds (decaying insects), and other nitrogenous substances in the water; but in no case did he find that free nitrogen from the atmosphere could serve as the source of nitrogen. While it is difficult to say with what organisms Morren worked, it is altogether probable that members of the *Volvocaceæ* were present among his "green," "brown," and "red infusoria."

No additional contribution to the subject, so far as the author is aware, was made until the appearance of Frank's paper (9) in 1888. In his investigation of the question of a possible fixation of free atmospheric nitrogen in natural soil without the instrumentality of cultivated plants, Frank exposed samples of unsterilized soil, poor in organic matter, in containers under a glass roof, watering them only with distilled water. During the 134 days that the experiment was continued, no phanerogams appeared, but in all cases the surfaces of the soil samples became covered with a thin, crustlike, greenish layer composed of "zwei spangrüne Oscillariaformen, die eine dick–, die andere sehr dünnfädig; ferner grünes Chlorococcum humicola, vielleicht auch Pleurococcus, sowie Vorkeimfäden von Moosen, also kryptogame Gewächse . . . Diatomaceen waren nicht zu finden." Analysis showed an undoubted increase in total nitrogen in the experiments. No increase in the nitrate content was observed,—the additional nitrogen being wholly in the form of organic nitrogenous compounds. These facts led the author to the conclusion that the abundance of algal cells, which are rich in protoplasm and therefore in organic nitrogen, accounts for the presence of the increased nitrogen in an organic form. That the appearance of the nitrogen in an organic form (algal substance) does not represent the primary fixation of free nitro-

gen and that the latter depends on an inorganic process, the inorganic compounds thus produced being subsequently assimilated by the algæ, is not rendered probable by later experiments. In these, Frank exposed samples of soil, kept free from vegetation, for long periods of time and at various temperatures. Plant growth was prevented by leaching the samples daily with hot water. In this manner any traces of nitrogen compounds formed were also obtained. Only at high temperatures—too high for plant growth—did he find a slight increase in total nitrogen and therefore believes that this process is of no importance under conditions which admit of plant growth. From these observations Frank concludes that the algæ themselves are the immediate agents in the fixation of free atmospheric nitrogen and inclines to extend this faculty to green plants in general.

In the same year, Gautier and Drouin (11) ascribed an entirely different function to soil algæ. Samples of artificial soils, free from organic material and containing only ammoniacal nitrogen, were exposed in a sheltered position for a considerable period of time. During the progress of the experiments the soil became more or less covered with a layer of green algæ (*Pleurococcus vulgaris, Protococcus viridis,* etc.). Analysis showed, in every case, a loss in total nitrogen, an even greater loss in ammoniacal nitrogen, and an intermediate gain in organic nitrogen. The authors assumed that the nitrogen lost was in the form of ammonia and that the amount of nitrogen appearing in the organic form was that part of the escaping ammoniacal nitrogen which, in bathing, so to speak, the algal cells on the surface, was absorbed, and subsequently built into organic nitrogen compounds. In support of this hypothesis the authors state that in proportion to the intensity of the algal growth loss in total nitrogen was diminished, and the amount of ammoniacal nitrogen converted into organic nitrogen increased. Gautier and Drouin thus looked upon the algæ as fixers of gaseous ammonia, which the soil tends to give off constantly, rather than as direct agents in the fixation of free atmospheric nitrogen.

In 1889, Frank (10) made the fixation of elementary nitrogen by soil-inhabiting algæ the subject of a special investigation. Four flasks containing sand moistened with distilled water and

plugged with cotton were treated as follows: Two were at once placed in the light; the third was covered with black paper and without further treatment placed with the first two; the fourth was exposed for six hours to a temperature of 100°C. and then placed with the rest. In the first two, rich algal growths developed, composed of two species of *Oscillatoria*, a blue-green "*Nostoc*–Form," a yellowish green "*Nostoc*–Form," a yellowish to pure green *Microcystis*, and a *Glœocapsa*. In the third and fourth flasks no growth of any kind developed. Analyses demonstrated that the total nitrogen content in the first two flasks had been doubled, whereas that in the latter two had suffered a distinct loss. The experiments were repeated with unsterilized soil, all air gaining access to the flasks being first passed through sulphuric acid to remove any ammonia present. The same characteristic algal flora developed and analysis again showed a decided increase in total nitrogen. On the basis of these experiments, Frank makes the generalization that the soil, as such, is unable to fix free atmospheric nitrogen, and that when the process does take place, it is effected by means of the vegetation of low algæ which develop in the soil, and which possess the ability of assimilating free gaseous nitrogen into vegetable, nitrogen-containing compounds. He goes still farther and states that the fact that low algæ utilize free nitrogen makes it more and more probable that the assimilation of elementary nitrogen is a faculty appertaining to the entire plant world provided with chlorophyll, and that, since the simple algal cell is endowed with this faculty, the thought is justified that the assimilation of free atmospheric nitrogen is as absolute and fundamental a process of the entire plant kingdom as is the assimilation of carbon dioxide.

Prantl (27), in cultivating fern prothallia in solutions with and without combined nitrogen, observed that whereas an abundant algal vegetation appeared in the former, only an *Anabæna*, or a *Nostoc*, grew in the latter. When placed in nitrogen-free media, the blue-green alga always grew abundantly. From this observation, and without analytical data, Prantl assumes that free-nitrogen assimilation had taken place, either a direct one by the alga, or an indirect one in which the alga assimilated the ammonium nitrite which, according to the theory of Schoenbein,

is formed in the vaporization of water. Of interest are the observations by the same author on the unicellular grass-green algæ, which he was unable to cultivate in solutions free from combined nitrogen. To these, therefore, he assigned the power of elementary-nitrogen fixation in a much smaller degree than to *Nostoc*.

Frank's conclusions were confirmed by the work of Schloesing and Laurent (31). These investigators supplemented the usual indirect method of analyzing the soil and harvest, with the direct method of determining at the beginning and at the end of the experiment the composition of the atmosphere in which the plants had been growing. To 2000 or 2500-gram quantities of a poor sandy soil 2.5 grams of limestone, 5 grams of a mixture of several rich soils, and a certain volume of a mineral nutrient solution containing, in some cases, a little potassium nitrate were added, and the whole placed in large flasks. In some, seeds of Jerusalem artichoke, oats, peas, and tobacco were planted; others, to be used as checks, remained unplanted. To each flask were added 5 cc. of a liquid obtained by diluting 5 grams of rich soil with 20 cc. of water. After fourteen weeks, during which time the seeds germinated and produced plants, the direct analytical method, confirmed by the results obtained by the indirect method, showed, except in two checks, an absorption of free atmospheric nitrogen. But the surfaces of the soils, during the progress of the experiments, became covered with green, cryptogamic plants, among which were mosses (*Bryum*, *Leptobryum*), and algæ (*Conferva*, *Oscillatoria*, *Nitzschia*). This fact led the authors to repeat the first series of experiments, in every case suppressing the growth of chlorophyllous cryptogams by covering the soils with a thin layer of dry, calcined, quartz sand. No trace of algæ or mosses appeared, and, except in the case of the peas, no absorption of free atmospheric nitrogen was observed. This fact, together with the evident fixation of nitrogen in the checks of the first series (in which an abundant chlorophyllous cryptogamic vegetation but no phanerogamic vegetation developed), and the absence of fixation in those checks in which little or no algal growth developed, led Schloesing and Laurent to conclude that there are some "inferior green plants" which are able to utilize free atmos-

pheric nitrogen. In the same year, Gautier and Drouin (12) reasserted their former conclusion as to the rôle of algæ in nitrogen fixation, holding that the methods of those who adhere to the opinion that algæ fix free nitrogen are too faulty to make conclusions drawn from them convincing.

In the work reported by Schloesing and Laurent in 1892 (32, 33) an attempt was made to reduce the complexity of the algal cultures by introducing into a single experiment only one or at most a few species of the algæ. All cultures were made on 600-gram quantities of either a subsoil or quartz sand to which was added (except in the two checks) a small quantity of an infusion prepared from soils. The cultures were allowed to develop for from three to six months, and, as in the previous experiments of these authors, analyses were made both of the contained atmosphere and of the soil and algal growth. The chlorophyllous plants which appeared in the various cultures are described as follows: I and II—essentially a mixture of *Nostoc punctiforme* Hariot and *Nostoc minutum* Desmazières, with a few colonies of *Cylindrospermum majus* Kuetz.; III—almost a pure culture of *Nostoc punctiforme;* IV—*Nostoc punctiforme* (less pure than in III), one colony of *Phormidium papyraceum*, and a small quantity of *Nostoc minutum;* V—two mosses—*Brachythecium rutabulum* and *Barbula muralis;* VI—an almost pure culture of an *Oscillarieæ* and *Microcoleus vaginatus*, with traces of *Tetraspora*, *Protococcus*, *Stichococcus*, *Ulothrix*, and *Lyngbya;* VII and VIII—checks with no growths, or at most a few small patches of *Phormidium autumnale* Gomont and *Nostoc punctiforme*. Both analytical methods showed abundant nitrogen fixation in the first four cultures but not an appreciable one in the fifth,—a fact which the authors explain on the basis of specific differences in plants in their ability to fix free atmospheric nitrogen. The checks showed no appreciable fixation. Separate analyses were made of the top-soil layers, containing the algal growths, and the deeper layers, the increased nitrogen being found in the algal stratum,—a fact which the authors consider important in proving that the algæ were responsible for the free-nitrogen fixation. In conclusion, Schloesing and Laurent admit the possibility that the bacteria present in the cultures had something to do with the fixation of free

nitrogen, and state that it is not possible to affirm with certainty that the algæ, free from other organisms, are able to effect fixation. Having observed, however, but few bacteria in the cultures they conclude that the algæ after all are the active agents in the fixation of elementary nitrogen.

Similar results were obtained by Koch and Kossowitsch (17). Sixty grams of washed, calcined sand were placed in large Erlenmeyer flasks and moistened with a mineral nutrient solution free from combined nitrogen. Since previous experiments had shown that algæ do not grow on sand free from combined nitrogen, 0.04 gram of calcium nitrate dissolved in 50 cc. of water were added to each flask. After inoculation with a suspension of algal cells obtained from heaps of lime, a continuous slow stream of air, washed in sulphuric acid, was passed through all the flasks. Three cultures were placed in a north window, three in the dark (to determine whether the bacteria contained in the cultures fixed free nitrogen), and the remainder were used in determining the initial total nitrogen. After fifteen weeks, during which time a rich algal vegetation[1] developed on all cultures exposed to the light, the contents of the flasks were analyzed *in toto*. Those exposed to the light showed an undoubted increase in total nitrogen, whereas those in the dark showed a slight loss in each case. Of particular interest was one culture which was brought into the light after it had remained in the dark for a considerable length of time. After the removal, a moderate growth of algæ appeared, and analysis showed a slight gain in total nitrogen, which, however, was less than that found in the cultures which had been exposed to the light during the entire period. In agreement with the earlier workers, these authors ascribed to algæ the faculty of free-nitrogen fixation, and emphasized the observation that the extent of this fixation was directly proportional to the intensity of the algal development. Petermann (26) reached a similar conclusion on the basis of experiments conducted on sterilized and unsterilized soils, which were respectively inoculated and uninoculated with algæ. The former in each case showed a distinct gain in nitrogen, whereas the latter showed either no increase or a slight loss.

[1] The authors failed to state what algæ developed, merely mentioning the presence of green and blue-green forms.

Incidental to his work on the respiratory quotient in algæ, Schloesing (30) reported that in a culture containing principally *Protococcus vulgaris* Ag., and smaller quantities of *Chlorococcum infusionum* Menegh., *Ulothrix subtilis* Kütz., and *Scenedesmus quadricauda* Bréb., there was at the end of two months no diminution of nitrogen in the supernatant atmosphere. This fact led the author to place these algæ among those forms which do not fix free atmospheric nitrogen.

As will have been observed, the work reported upon in the contributions cited was done with impure cultures. While in some cases but a single species was used, bacteria were present in all cases. Although in many instances this is not expressly stated, the author's experience convinces him that the technique employed by these earlier workers made the contamination of their cultures with bacteria very probable. It is evident, therefore, that in the work done thus far it is impossible to state with certainty whether the results obtained are due to the activity of the algæ, or to the bacteria, or to both.

The first work done on the fixation of free nitrogen by algæ in which pure cultures were used was that of Kossowitsch (18), in 1894. The only form isolated in pure culture by this investigator was one which he states resembled both *Cystococcus* (Nägeli) and *Chlorella vulgaris* Bey. He leaves its identity uncertain but designates it, for convenience, *Cystococcus*. Preliminary experiments with impure cultures of this alga had demonstrated that asparagin and ammonium tartrate could not serve as the source of nitrogen and that growth took place only when nitrates were supplied. In the experiments with pure cultures, flasks containing 70 grams of clean sand moistened with a mineral nutrient solution containing a known amount of calcium nitrate were inoculated with a carefully tested pure culture of *Cystococcus* and allowed to remain four months. To a number of the cultures dextrose was added, and to others, in addition to this sugar, pea-tubercle bacteria. At the conclusion of the experiments the cultures were carefully tested for purity. Analysis in every case showed an absence of free-nitrogen fixation, and demonstrated clearly for the first time that an alga, *Cystococcus*, under the conditions realized in the experiment, did not fix free atmospheric nitrogen. That the

same holds true for this alga in nature seemed probable to Kossowitsch, who found that it grew vigorously only so long as a nitrate was present. He further observed that after growth had ceased in any culture, it was promptly resumed upon the addition of a nitrate solution, but not when the nitrogen-free nutrient solution was added. Similar cultures were started in which the inoculation material was either a mixture of algæ and bacteria derived from soil or lime, or a mixture of soil bacteria with a pure culture of *Cystococcus*. In each case the cultures were set up with and without dextrose. Table I gives the results of these experiments.

TABLE I

RESULTS OF KOSSOWITSCH'S EXPERIMENTS WITH PURE AND MIXED CULTURES

+ or − Sugar	Content of cultures	Mg. of N in cultures	
		Initial	Final
−	*Cystococcus* (pure culture)	2.6	2.7
+		2.6	2.7
−	*Cystococcus*, *Phormidium*, soil bacteria, moulds	2.6	7.1
+		2.6	9.5
−	Pure *Cystococcus* culture and bacteria	2.6	3.1
+		2.6	8.1
−	*Stichococcus* and bacteria	2.6	2.3
+		2.6	2.7
−	*Nostoc*, large round alga, *Scenedesmus*, soil bacteria	2.6	?
+		2.6	19.1
−	*Nostoc*, a *Cylindrospermum* (small form), soil bacteria	2.6	8.8
+		2.6	25.4

Cystococcus, in pure culture, was again unable to fix free gaseous nitrogen, and the same conclusion is reached by Kossowitsch for *Stichococcus*, which even in the presence of a mixture of bacteria failed to fix elementary nitrogen. Of especial interest are the cultures of pure *Cystococcus* with bacteria, as in these the fixation is ascribable only to the bacteria. Which of the organisms in the remaining cultures are responsible for

the marked fixation of free atmospheric nitrogen it is impossible to say, the author states. However, from his own results, and those of previous investigators, that the presence of algæ exercises a favorable effect on the process of free-nitrogen fixation, and, further, that the algæ thus far studied in pure culture do not possess this faculty of fixation, Kossowitsch concludes that the algæ play an indirect rôle. He believes they do this by furnishing, through their photosynthetic activity, carbohydrates to the nitrogen-assimilating bacteria. He would look upon the algæ as occupying the same position with reference to free-living, nitrogen-fixing bacteria as the legumes do with reference to the nodule organisms.

Stocklasa (35), while not making his conclusion very clear, leads one to believe that he considers certain algæ (which he fails to enumerate) capable of fixing free atmospheric nitrogen. Unfortunately, all of Stocklasa's experiments were carried out with impure cultures. Molisch (23), in conducting experiments with algæ relative to the necessary nutrient elements, attempted to cultivate *Microthamnion Kützingianum* Näg., *Stichococcus bacillaris* Näg., *S. major* Rbh., *Ulothrix subtilis* (?) Kütz., and *Protococcus sp.*—all in impure culture—on a nitrogen-free mineral nutrient solution. In every case the algæ failed to grow, and Molisch was led to the conclusion that algæ require combined nitrogen for their development. Although no experiments in which combined nitrogen was furnished to the algæ were conducted, the author nevertheless makes the statement, based principally on the work of Kossowitsch just reviewed, that algæ are not able to fix free atmospheric nitrogen.

In the next year Bouilhac (4) reported that he had succeeded in isolating in pure culture *Schizothrix lardacea, Ulothrix flaccida,* and *Nostoc punctiforme.* Unfortunately, this author does not give a detailed account of his isolation methods. Six flasks containing a mineral nutrient solution free from combined nitrogen were inoculated with each alga, and to three of each a drop of soil suspension was added. No growth whatever developed in any of the *Schizothrix* and *Ulothrix* cultures, nor in the *Nostoc* cultures to which the suspension had not been added. But in those cultures of the latter to which a drop of soil suspension had been added, a splendid growth appeared and in each

culture analysis showed a nitrogen fixation of from 11 to 23 milligrams. From a second series (in which the cultural solution contained per liter 0.1 gram arsenic acid in the form of potassium arsenate) a similar result was obtained, with fixation of nitrogen of from 5 to 60 milligrams. The presence of *Ulothrix* or *Pleurococcus* in addition to the *Nostoc* and bacteria seemed to have no appreciable effect on the quantity of nitrogen fixed. Bouilhac thus concluded that *Schizothrix lardacea* and *Ulothrix flaccida* (either alone or in the presence of soil bacteria) and *Nostoc punctiforme* (in the pure state) are unable to fix free atmospheric nitrogen in the absence of combined nitrogen. The abundant fixation in the cultures containing a mixture of *Nostoc* and soil bacteria is not ascribed by the author to the activity of either organism alone.

Richter (28) observed pots of soil with and without plants, some placed in the dark, others in the light. While a rich algal vegetation developed in the latter, none appeared in the former. Only in a few cases was the growth accompanied by a marked free-nitrogen fixation, but in these instances the author believes it due to the algæ. Pure cultures were not employed. Benecke (1) contributed some observations made on cultures of *Hormidium*, *Vaucheria*, *Cladophora*, and members of the *Conjugales*,—all containing bacteria. In nitrogen-free cultures there appeared what Benecke termed "nitrogen-hunger," a condition which is characterized in *Hormidium* by the production of very long, pale filaments, the cells of which become extremely long and in which the development of the chloroplast is so meager that the cells are almost colorless. Stocklasa (36) found that the "Alinit" bacteria fix free gaseous nitrogen in much larger quantities when grown in the presence of species of *Stichococcus* and *Nostoc*. This influence he considers to be due to the pentosans which, according to his belief, are present in large quantities in various algæ, and which, because of their ready solubility in water, serve as a favorable energy-furnishing medium for free-nitrogen-fixing bacteria.

A noteworthy contribution to the subject is that of Krüger and Schneidewind (19). These authors for the first time conducted extensive experiments with a variety of algæ in pure culture, including *Stichococcus chloranthus*, *S. major*, *S. bacil-*

laris, and *S. sp.*, the latter isolated from five different sources; *Chlorella sp.*, from the group of which *Chlorella vulgaris* Bey. is typical (also isolated from five different localities); *Chlorella protothecoides* and three other isolations of a form or forms belonging to the same group; *Chlorothecium saccharophilum* and five other isolations of forms belonging to the same group; and lastly, *Cystococcus humicola*. The media employed by the authors included the following:

1. One per cent dextrose, 0.2 per cent K_2PO_4, 0.04 per cent MgSO4, 0.02 per cent $CaCl_2$, and 1 drop of a 2 per cent $FeCl_3$ solution to each 100 cc. of solution.
2. Ignited sand moistened with solution 1.
3. Solution 1 plus 0.25 per cent $(NH_4)_2SO_4$, and 0.25 per cent $NaNO_3$.
4. Ignited sand moistened with solution 3.
5. One-half per cent beef extract, ½ per cent peptone, and ½ per cent dextrose.
6. Ignited sand moistened with solution 5.
7. Diluted beerwort.
8. Ignited sand moistened with solution 7.
9. Humous clay soil plus 35 per cent sand moistened with distilled water.

The results obtained were uniform in that the media, free from combined nitrogen, failed to produce a healthy growth, whereas those containing nitrogen in a combined form showed an abundant growth,—some of the algæ preferring the nitrogen in an organic and others in an inorganic form. Further, no fixation of free atmospheric nitrogen was noted in any of the cultures. Krüger and Schneidewind conclude that there is a strong probability that all other chlorophyllous soil algæ of this kind are unable to fix free atmospheric nitrogen, and, in general, agree with the opinion of Kossowitsch that the soil-inhabiting algæ supply the free-living, nitrogen-fixing organisms with the necessary non-nitrogenous, energy-furnishing material.

Conclusions similar to those of Kossowitsch were reached by Deherain and Demoussy (8), who succeeded in cultivating blue lupines free from root nodules in humus-free sand, the surface of which became covered with *Phormidium autumnale* and *Ulothrix flaccida* in the course of the experiments. The authors

explained the growth of the lupines by supposing that the soil bacteria fixed free nitrogen at the expense of energy-furnishing organic materials supplied by the algæ, and that the nitrogen so fixed in organic form became available to the legumes.

A return to the conclusion that members of the *Cyanophyceæ* fix free atmospheric nitrogen is found in an investigation by Beyerinck (2). From 1½ to 2-liter portions of tap or distilled water containing 0.02 per cent dipotassium acid phosphate were inoculated with 1–2 grams of garden soil, and placed in the light. After several weeks a characteristic growth of blue-green algæ developed, containing, among other species, *Anabæna catenula*, a form related to or identical with *Nostoc paludosum*, and *Nostoc sphæricum*,—all non-motile species of *Cyanophyceæ*. The development of the blue-green algæ in an almost nitrogen-free medium led Beyerinck, without analytical data, and in spite of the evident contamination of his cultures with soil bacteria, to the conclusion that the *Cyanophyceæ* belong to the class of organisms possessing the faculty of free-nitrogen fixation. He regards the *Cyanophyceæ* as the only known organisms capable of synthesizing their organic materials from carbon dioxide and free nitrogen, and considers as significant in this connection the observations of Graebner (13) and Treub (37), who found that in the sequence of floras on fresh sand and lava soils, species of *Cyanophyceæ* are the first to appear.

Cystococcus humicola was once more subjected to a careful investigation by Charpentier (7). His previous experiments had demonstrated that the dry weight of algal growth obtained in liquid glucose media was about one-half that of the weight of glucose consumed, and that 5.14 per cent of this dry weight was nitrogen. He then pointed out that the quantity of nitrogen furnished by Kossowitsch to his pure cultures of *Cystococcus humicola* in the form of potassium nitrate was sufficient to produce at least 40 milligrams of growth (dry weight), and that while this growth was being produced it might not be necessary for the alga to seek nitrogen from the atmosphere. Once the dextrose was exhausted, the alga might, it is true, develop at the expense of atmospheric carbon dioxide, but the author holds the opinion that this would mean a double expenditure of energy for the assimilation of both carbon dioxide and free nitrogen and

that under these conditions growth would be difficult. Because of the vast amount of energy necessary for free-nitrogen fixation, as illustrated by *Clostridium Pasteurianum*, the author suggests that there is a strong probability that *Cystococcus* is capable of assimilating free nitrogen only when the expenditure of energy in carbon assimilation is reduced to a minimum,—that is to say, when abundant available organic materials are furnished. He further emphasizes the necessity of employing combined nitrogen in a less readily available form than nitrates, suggesting organic nitrogenous compounds. On media composed of a decoction of beans to which were added 1 per cent and 2 per cent of dextrose and gelatin, respectively, *Cystococcus* was grown and the entire culture analyzed for total nitrogen. Although care was taken to have an abundance of available organic material (dextrose) present, Charpentier found that in no case was there any indication of free-nitrogen fixation. He further found that ammonia, asparagin, and peptone were each able to serve as the sole source of nitrogen.

The association of blue-green algæ and soil bacteria is again referred to as an effective agent in free-nitrogen fixation by Bouilhac and Giustiniani (5, 6). Buckwheat, white mustard, corn, and cress were planted in clean sand moistened with a mineral nutrient solution free from combined nitrogen, and the substrata inoculated with *Nostoc punctiforme* and *Anabæna sp.* covered with bacteria. The phanerogams grew to maturity and analysis showed a marked fixation of free atmospheric nitrogen.

Of particular interest are the observations of Heinze (14), who, however, fails to state whether or not the *Chlorella* experimented with was in pure culture. He found that no appreciable growth took place in cultural solutions free from combined nitrogen, but that in the presence of the latter a rich growth appeared, unaccompanied, however, by a definite fixation of nitrogen. More important are his experiments with *Nostoc* in impure condition, a good growth of the form being obtained in a mineral nutrient solution free from combined nitrogen and sugar. These cultures, as well as others on soil inoculated with a similar *Nostoc* culture contaminated with bacteria and fungi, showed a definite amount of free-nitrogen fixation. Heinze was unable to find *Azotobacter* present, and this, together with the observa-

tion that the contaminating fungus in pure culture was unable to fix free nitrogen, led the author to the conclusion that the *Nostoc* is, in all probability, directly responsible for the free-nitrogen fixation. Further, he would place *Azotobacter* in close relationship with the *Chroococcaceæ*, a family in which, he suggests, some forms capable of fixing free atmospheric nitrogen may be found.

Richter (29), working with pure cultures of *Nitzschia palea* and *Navicula minuscula*, reached the conclusion that the former, and probably the latter also, is unable to assimilate elementary nitrogen in the absence of combined nitrogen. Heinze (15), in experimenting with a *Nostoc* culture which he had purified until it contained as a contamination only a *Streptothrix*, found that in solutions free from combined nitrogen and sugar but containing respectively mono, di, and tripotassium phosphate, a clearly demonstrable amount of free atmospheric nitrogen was fixed. The *Streptothrix* was subsequently isolated and tested as to its ability to fix elementary nitrogen, both with and without sugar, but always with negative results. In conclusion, Heinze reasserts his former belief that *Nostoc* is capable of fixing elementary nitrogen.

Mameli and Polacci (22) succeeded in growing *Oedogonium*, *Spirogyra*, *Zygnema*, and *Protococcus* in nutrient solutions free from combined nitrogen, and demonstrated by analysis an increase in total nitrogen. They ascribed to these forms, and to chlorophyllous cells in general, the faculty of synthesizing ammonia from free nitrogen and nascent hydrogen. Pure cultures were not used. Boresch (3) found that *Phormidium corium* Cohn became brown when grown in solutions containing very small amounts of combined nitrogen, but that the green color reappeared following the addition of potassium nitrate or organic nitrogen compounds. Several species of *Oscillatoria*, *Rivularia*, and *Chroococcus* behaved similarly. But *Anabæna sp.* did not change color even when the solution in which it was growing had become completely exhausted of its combined nitrogen. While the investigation concerns itself primarily with the relation of nitrogen to the color in algæ, the observations point once more to species of *Anabæna* as possibly belonging to the class of free-nitrogen-fixing organisms, and

equally clearly to the conclusion that the remaining forms experimented with do not belong to this class.

Oes (25) made the observation that *Azolla* with its endophytic *Anabæna Azollæ* grew exceedingly well in mineral nutrient solution free from combined nitrogen. Analysis showed a distinct fixation of nitrogen. Attempts to cultivate the *Anabæna* in pure culture failed. While calling attention to the possible direct rôle of the associated bacteria in the observed fixation, the author inclines to the view that *Anabæna Azollæ* is itself capable of fixing free atmospheric nitrogen.

The preceding survey of literature shows that in all of the earlier investigations, and in a considerable number of the later ones, impure cultures were used. In experiments conducted under these conditions, it is evident that negative results are, in general, more reliable than positive ones. Attention should therefore be called to the negative results which have been obtained from investigations with impure cultures. These, as will be seen from the literature cited, include a large number of genera and species from both grass-green and blue-green algæ, and indicate in many cases with a reasonable degree of certainty that the faculty of elementary-nitrogen fixation is absent in a very considerable number of species of both the *Chlorophyceæ* and *Cyanophyceæ*. In the former class, all investigations conducted with pure cultures have led without exception to the conclusion that these forms are unable to fix free atmospheric nitrogen.

As regards the *Cyanophyceæ*, it should be stated at the outset that while many observations are on record both affirming and denying free-nitrogen fixation in the group, it is questionable whether experiments have been conducted with more than a single species in pure culture. Bouilhac, it is true, claims to have isolated *Schizothrix lardacea* and *Nostoc punctiforme* in pure culture. From the meager account given of the isolation technique, it appears very improbable that the latter form was actually obtained in culture free from bacteria, although the former may have been. However, the work of Heinze, while not conducted with pure cultures, renders free-nitrogen fixation in *Nostoc* probable, and it appears especially desirable, there-

fore, to study representatives from this genus as well as other members of the group *Cyanophyceæ*.

In the progress of the work about to be reported, it soon became obvious that the development of pure culture methods would constitute a very considerable portion of the investigation, and it was deemed advisable to limit the nitrogen phase of the problem to the fixation of atmospheric nitrogen in the complete absence of combined nitrogen, leaving for a subsequent report the problem of elementary-nitrogen fixation in the presence of combined nitrogen. The work concerning pure culture methods will be found reported elsewhere (34).

Experimental

Materials and Apparatus

Most of the algæ isolated were soil-inhabiting species. Preliminary experiments showed that in nearly every case better growth was obtained on solid media than in liquid ones. For this reason it was decided to conduct all experiments concerning the fixation of free nitrogen in the complete absence of combined nitrogen on a solid medium. Agar could not be sufficiently freed from all traces of combined nitrogen, and the difficulties involved in preparing large quantities of silicic acid jelly sufficiently pure were so great that a No. 2½ ground quartz was finally decided upon.

Preparation of the Sand.—The sand, after being thoroughly washed, was boiled in concentrated hydrochloric acid for two hours, subsequently washed free from chlorides with distilled water, and then heated almost to dull redness for from four to five hours. The sand was then boiled a second time in chemically pure concentrated hydrochloric acid and again washed with distilled water until chlorides could no longer be detected. When this stage had been reached the washing with distilled water was continued a dozen times more, after which the sand was drained as thoroughly as possible and the washing completed with from five to ten changes of nitrogen-free water. After drying the sand in a clean evaporating dish, a sample was boiled in nitrogen-free water and the liquid tested for ammonia, nitrites and nitrates, but only uniformly negative results were obtained.

Nitrogen-free Water.—The distilling apparatus used was, in general, like that described by Jones and Mackay (16) for the preparation of water with a very low electrical conductivity, except that the water was triply distilled in place of doubly, and from glass throughout in place of being condensed in a block tin tube. Fig. 1 represents the distilling apparatus, and it need only be pointed out that flask III was added to obviate any possibility of contaminating the distillate with spray from flask II. The water obtained from this still gave uniformly negative results when tested for ammonia, nitrites and nitrates.

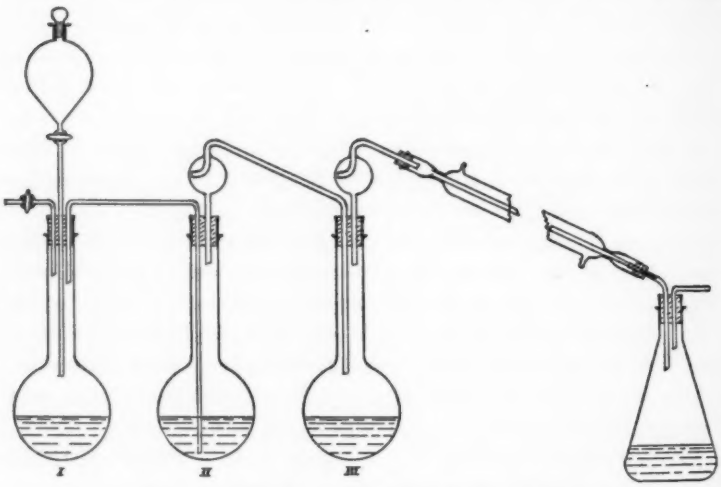

Fig. 1. Distilling apparatus for nitrogen-free water

Cultural Apparatus.—One hundred cc. flasks, carefully cleaned in acid-dichromate cleaning mixture, rinsed in nitrogen-free water and dried, were connected in series of ten each in the 1912 experiment (eight in the 1913 experiment) by means of glass tubing and rubber stoppers as shown in pl. 3 fig. 1. The glass tubing was cleaned in the same manner as the flasks, and the rubber stoppers were boiled in dilute alkali, then in dilute hydrochloric acid, and subsequently washed with distilled and nitrogen-free water. Into each flask of the 1912 experiment an accurately weighed 40-gram quantity (in the 1913 experiment

30 grams) of sand was placed. For purposes of aëration the separate series of flasks were joined together in groups of five, as shown in pl. 3 fig. 2, and the free end of the common connecting tube provided with three sets of triple wash-bulbs, —the two nearest the flasks containing nitrogen-free water, which served to moisten the air after passing through the third bulb containing 25 per cent sulphuric acid. In order to aërate any particular series of flasks it was only necessary to attach a filter pump to the rubber tube at the end of the series which it was desired to aërate and to open the pinchcock until the desired stream of air passed through the wash-bulbs.

Chemicals.—The inorganic compounds used were all Baker and Adamson's analyzed chemicals; the organic compounds were Merck's highest purity chemicals.

Cultural Solutions.—In the 1912 experiment, in which each series contained ten flasks, the following ten cultural solutions were used and in the following order, the flasks being numbered correspondingly:

1. NH_4NO_3 0.5 grams,
   $MgSO_4.7H_2O$ 0.2 grams,
   K_2HPO_4 0.2 grams,
   $CaCl_2.?H_2O$ 0.1 grams,
   $FeSO_4$ trace,
   Nitrogen-free water 1000 grams.
2. The same as No. 1, but containing 0.250 grams of NH_4NO_3 instead of 0.5 grams.
3. The same as No. 1, but containing 0.100 grams of NH_4NO_3 instead of 0.5 grams.
4. The same as No. 1, but containing 0.050 grams of NH_4NO_3 instead of 0.5 grams.
5. The same as No. 1, but free from combined nitrogen.
6. The same as No. 5, but containing 2 per cent *d*-glucose.
7. The same as No. 3, but containing 2 per cent *d*-glucose.
8. The same as No. 5, but containing 2 per cent mannite.
9. The same as No. 3, but containing 2 per cent mannite.
10. The same as No. 3, but containing 2 per cent saccharose.

In the 1913 experiment, in which each series contained eight flasks, the following eight cultural solutions were used:

1. The same as No. 5 in the 1912 experiment.
2. The same as No. 1 in the 1912 experiment.
3. The same as No. 5 in the 1912 experiment, but with 5.26 grams of *d*-glucose (making a glucose solution isotonic with a 1 per cent saccharose solution) added.
4. The same as No. 1 in the 1912 experiment, but with 5.26 grams of *d*-glucose (making a glucose solution isotonic with a 1 per cent saccharose solution) added.
5. The same as No. 5 in the 1912 experiment, but with 5.32 grams of mannite (making a mannite solution isotonic with a 1 per cent saccharose solution) added.
6. The same as No. 1 in the 1912 experiment, but with 5.32 grams of mannite (making a mannite solution isotonic with a 1 per cent saccharose solution) added.
7. The same as No. 5 in the 1912 experiment, but with 10.00 grams of saccharose (making a 1 per cent saccharose solution) added.
8. The same as No. 1 in the 1912 experiment, but with 10.00 grams of saccharose (making a 1 per cent saccharose solution) added.

The solutions containing the organic compounds were all made isotonic in order to obviate possible differences in growth due to different osmotic pressures of the cultural solutions.

The exact volume of solution necessary to just saturate the amount of sand used in each flask was determined and this amount of the various solutions added to the corresponding flasks. The stoppers were then lightly inserted and one group sterilized at a time in a large Kny-Scheerer horizontal autoclav, at six pounds pressure for one and one-quarter hours.

INOCULATION

The groups of flasks were transferred directly from the autoclav to the inoculating room which had previously been "steamed down." All inoculations were made with a DeVilbis atomizer, which, with the exception of the bulb, is made of metal and glass throughout. The bulb was removed and the opening of the metal tip, to which the former is attached, plugged with cotton. After filling the glass container one-half full of solution No. 5 (1912 series), the whole (with the exception of the bulb)

was sterilized. After cooling, the liquid was inoculated with the desired organism (care being taken to avoid introducing any agar, which can readily be done if hard agar cultures are used from which to make the inoculation). The DeVilbis atomizer is provided with an adjustable metal tip so that the spray may be directed downward. The metal tip further admits of sterilization by flaming. By exercising care and keeping the hands moist with alcohol, comparatively few contaminations result, only four having appeared in a total of 320 inoculations.

Attention should be called to the importance of inoculating in such a way that an approximately equal number of organisms are introduced and that they are uniformly distributed over the substratum. Unless this is done growth comparisons cannot be made with any considerable degree of accuracy, as differences may be due to localized and unequal inoculation. This is especially true in algæ which do not form motile cells and which, therefore, are unable to spread rapidly over the substratum.

GROWTH AND OBSERVATIONS

All groups of flasks of the 1912 experiment were placed in the light of north windows at the ordinary room temperature and the cultures aërated at intervals of from three days to a week.

The 1913 experiment was set up in duplicate, one-half being placed in a glass incubator kept constantly at from 29.5 to 30.5° C., and the other half in a similar incubator at the ordinary room temperature. Both series of cultures were placed directly in front of a north window and were aërated from time to time.

Space will not permit the detailed tabulation of the observations on growth. In the following tables, growth is indicated without reference to time. A few general statements may, however, serve to give some idea as to the relation of the composition of the cultural medium to the time elapsing before a macroscopically visible growth appeared. In almost every case, growth was observed first on the glucose-containing medium and almost as soon or slightly later on the one containing saccharose. It should be said, however, that a healthy growth was maintained on these two media, in most cases, for but a short time. *Chlamydomonas pisiformis* Dill forma *minor* Spargo is a marked exception in this respect, a splendid, healthy

TABLE II

RESULTS OF THE 1912 EXPERIMENT

(August 6, 1911—July 1, 1912.)

Organism	Sol. 1* (with comb. nitrogen)	Sol. 5 (without c.n.)	Sol. 6 (without c.n.)	Sol. 7 (with c.n.)	Sol. 8 (without c.n.)	Sol. 9 (with c.n.)	Sol. 10 (with c.n.)
Chlamydomonas pisiformis Dill forma *minor* Spargo	++	−†	−	++++	−	++++	++++
Chlorella sp., large form with clathrate chromatophore	+++	−	−	++	−	+++	++
Kirchneriella sp., a form without marked gelatinous envelope	+	−	−	+	−	+	+
Protosiphon botryoides (Kütz.) Klebs	++	−†	−†	+++	−	+++	+++
Chlorococcum humicola (Näg.) Rabenh.	+++	−	−	++++	−	+++	++++
Chlorella vulgaris Bey.	+++	−†	−	++	−	+	++
Stichococcus bacillaris Näg.	+++	−	−	++	−	−	−

Slight, fair, good, and splendid growths are respectively indicated by +, ++, +++, and ++++. No growth is indicated by −.

* The mention of growth in solutions 2, 3, and 4 is omitted because growth differences were not marked. These solutions were introduced in each series in order to note the effect of steadily decreasing quantities of ammonium nitrate on the intensity of growth.

† A scarcely detectable growth developed in these cases which, however, disappeared in all cases within a short time. The growth was so slight as to be noticeable only when the flasks were compared with others absolutely free from growth.

growth being maintained for a very long time. Growth on the mannite-containing medium was usually slower in making its appearance, but, in general, remained healthy for a longer period of time than those on glucose or saccharose. With very few exceptions, growth appeared last on the purely synthetic medium, but was maintained in a state of vigor longer

TABLE III

RESULTS OF THE 1913 EXPERIMENT

(March 29–April 16, 1913.)

Organism	Sol. 1 (without c.n.)	Sol. 2 (with c.n.)	Sol. 3 (without c.n.)	Sol. 4 (with c.n.)	Sol. 5 (without c.n.)	Sol. 6 (with c.n.)	Sol. 7 (without c.n.)	Sol. 8 (with c.n.)
Chlamydomonas pisiformis Dill forma *minor* Spargo. Temp. 18.5–24°C.	–	++	–	+++	–*	++	–	+++
Ditto. Temp. 29.5–30.5°C.	–	–	–	++	–	–	–	++
Chlorella sp., large form with clathrate chromatophore. Temp. 18.5–24°C.	–	+	–	++	–	+	–	+++
Ditto. Temp. 29.5–30.5°C.	–	+	–	++	–	–	–	++
Stichococcus bacillaris Näg. Temp. 18.5–24°C.	–	+	–	++	–	+	–	++
Ditto. Temp. 29.5–30.5°C.	–	–	–	++	–	–	–	++
Chlorococcum humicola (Näg.) Rabenh. Temp. 18.5–24°C.	–	+	–	++++	–	+	–	++++
Ditto. Temp. 29.5–30.5°C.	–	–	–	+++	–	–	–	+++
Protosiphon botryoides (Kütz.) Klebs. Temp. 18.5–24°C.	–	+	–	+++	–	++	–	+++
Ditto. Temp. 29.5–30.5°C.	–	–	–	+++	–	–	–	+++
Chlorella vulgaris Bey. Temp. 18.5–24°C.	–	+	–	++	–	+	–	++
Ditto. Temp. 29.5–30.5°C.	–	–	–	–	–	–	–	–
Kirchneriella sp. Temp. 18.5–24°C.	–	–	–	–	–	–	–	–
Ditto. Temp. 29.5–30.5°C.	–	–	–	–	–	–	–	–

*A scarcely detectable and rapidly disappearing growth developed as in the 1912 experiment.

than on any of the organic-compound-containing media. Glucose, saccharose, and mannite were chosen as energy-furnishing compounds because of their general usefulness in this capacity among free-nitrogen-fixing bacteria, and also because they are representatives from three great classes of carbon compounds.

Certain unpublished experiments, carried out by B. M. Duggar on nitrogen fixation in the fungi, indicate that, whereas no fixation takes place at ordinary temperatures, it does take place at elevated temperatures. It was thought desirable, therefore, to investigate the effect of elevated temperature on the process of elementary-nitrogen fixation by algæ in the absence of combined nitrogen. However, the results tabulated in table III show clearly that not only did no growth on any nitrogen-free medium appear at the higher temperature, but also that that appearing on nitrogen-containing media was, in many cases, poorer than that obtained in cultures kept at ordinary temperatures. It should further be noted that growth was in some cases entirely suppressed. It would appear, therefore, that in the species investigated, growth at elevated temperatures is less vigorous than at ordinary temperatures and that, in all probability, no favorable effect on free-nitrogen fixation is to be expected by growing these species at the higher temperature maintained in the experiment.

The incipient, ephemeral growth which was observed in a few cases where combined nitrogen was not furnished is believed to be due to the minute quantity of combined nitrogen which was unavoidably introduced in the inoculation process. The inoculating material was, of necessity, derived from agar containing ammonium nitrate, and while no agar was transferred it is altogether probable that enough combined nitrogen was carried over in the water adhering to the cells to account for the trace of growth. It should be emphasized again that in every case this growth was so slight as to have escaped detection had not a comparison been made with a flask absolutely free from growth.

In table IV the results of the two experiments are combined and show that in seven species complete results have been obtained. These results indicate with perfect uniformity that growth, under the conditions realized in the experiments, is impossible in the absence of combined nitrogen, even when readily

TABLE IV

SUMMATION TABLE OF 1912 AND 1913 EXPERIMENTS

(Solutions numbered as in 1913 experiment.)

Organism	Sol. 1 (without c.n.)	Sol. 2 (with c.n.)	Sol. 3 (without c.n.)	Sol. 4 (with c.n.)	Sol. 5 (without c.n.)	Sol. 6 (with c.n.)	Sol. 7 (without c.n.)	Sol. 8 (with c.n.)
Chlamydomonas pisiformis Dill forma *minor* Spargo	−	++	−	++++	−	++++	−	++++
Chlorella sp.	−	+++	−	++	−	+++	−	+++
Stichococcus bacillaris Näg.	−	+++	−	++	−	+	−	++
Chlorococcum humicola (Näg.) Rabenh.	−	+++	−	++++	−	+++	−	++++
Protosiphon botryoides (Kütz.) Klebs	−	++	−	+++	−	+++	−	+++
Chlorella vulgaris Bey.	−	+++	−	++	−	+	−	++
Kirchneriella sp.	−	+	−	+	−	+	−	+

assimilable energy-furnishing compounds like glucose, mannite, and saccharose are supplied; and that, therefore, these forms, under the conditions stated, are totally unable to fix free atmospheric nitrogen in the complete absence of combined nitrogen.

Conclusions

1. In agreement with all work that has previously been done on the assimilation of elementary nitrogen by grass-green algæ in pure culture, it has been found that *Chlamydomonas pisiformis* Dill forma *minor* Spargo, *Protosiphon botryoides* (Kütz.) Klebs, *Chlorococcum humicola* (Näg.) Rabenh., *Chlorella vulgaris* Bey., *Stichococcus bacillaris* Näg., *Chlorella sp.*, and *Kirchneriella sp.*, are unable to fix free atmospheric nitrogen in the complete

absence of combined nitrogen, under the conditions realized in the experiments.

2. A slightly elevated temperature (from 5 to 10° C. above the ordinary range of room temperature—18–24°C.) does not, as is the case in certain fungi, enable the algæ investigated to fix free gaseous nitrogen in the complete absence of combined nitrogen.

In conclusion, the author wishes to express his sincere appreciation and gratitude to Dr. George T. Moore, at whose suggestion the work reported upon in this paper was undertaken and under whose constant attention and generous aid it was carried to completion; to Dr. B. M. Duggar, for many valuable suggestions and innumerable courtesies; to Mildred Spargo Schramm, for kindly encouragement and help throughout the investigation; and to Dr. George R. Hill, Jr., for substantial aid during the last year of the work.

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EXPLANATION OF PLATE

PLATE 3

FIG. 1. Culture flasks containing quartz sand joined together in series of eight each, but before arrangement into groups.

FIG 2. Five series of culture flasks arranged in a group with a common connecting tube (on the left) and a series of three triple wash-bulbs. On the right, the rubber tubing, provided with pinchcocks, is shown attached to each series for use in aëration.

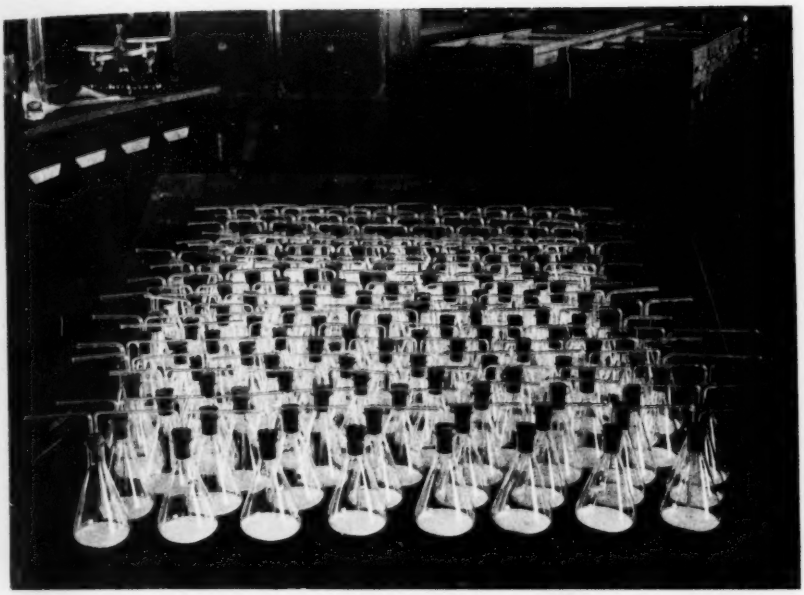

FIG. 1.

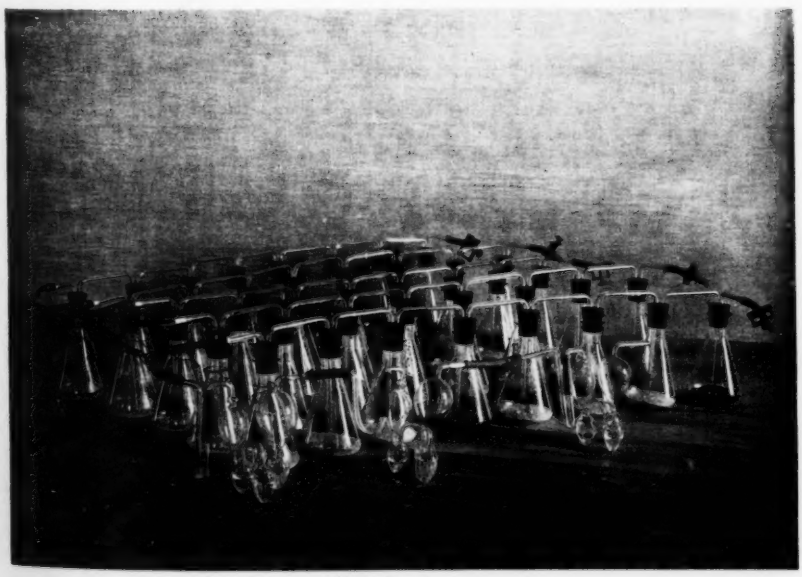

FIG. 2.

SCHRAMM — GREEN ALGAE AND ELEMENTARY NITROGEN

COCKAYNE, BOSTON.

THE THELEPHORACEÆ OF NORTH AMERICA. I[1]

EDWARD ANGUS BURT

Mycologist and Librarian to the Missouri Botanical Garden
Associate Professor in the Henry Shaw School of Botany of
Washington University

INTRODUCTION

This monographic study of the North American *Thelephoraceæ* was begun in 1894 as the author's contribution towards a greatly needed manual of the *Basidiomycetes* of the United States,—a need that still confronts us. It has been necessary to carry on these investigations in connection with college and other work which required most of my time, but the long period covered has been an advantage; for during these two decades there has been such widespread interest in the *Thelephoraceæ* on the part of American students of fungi that it has been possible to study this family and its distribution from extensive series of freshly collected specimens from all the important regions of North America with the exception of Alaska, Mexico, and the Colorado-New Mexico region of the United States, from which but small collections have been received. These specimens have been preserved unpoisoned in my herbarium in insect-proof tin boxes which receive herbarium sheets, and each will be cited by the number or other designation adopted by my correspondents in order that their specimens may be as useful for future reference as my own. The quantity of material always awaiting examination has confined my work to a systematic treatment of this family.

Except in the case of types of species, specimens of published exsiccati, and the specimens of Schweinitz's herbarium, I cite but few specimens from the large herbaria. This is done on account of the difficulty and large amount of time involved in making a study of the material contained in them. Serious changes in the condition of the specimens in these herbaria have been occasioned partly by time but more largely by the poisonous solutions with which the specimens were soaked for preservation under old-fashioned methods of herbarium procedure,—

[1]Issued July 1, 1914.

methods well enough adapted for flowering plants but not for fungi.

Early in the work it became apparent that the diagnoses of known species of resupinate *Thelephoraceæ* had failed utterly to enable the leading working mycologists of any country to recognize with certainty in the species about them those described in other countries, or those described for their own country by earlier students. The truth of this statement is shown by the errors and confusion in names of the common species which have been distributed in exsiccati, by the fact that in the large herbaria several different species are likely to bear the same specific name on the same or successive sheets, and by the vastly more important fact that the masters of mycology of each age, when relying wholly on the diagnoses published by their contemporaries or predecessors, have described as new species common and conspicuous resupinate fungi which had been accurately described by immediate contemporaries or predecessors, and in very many cases just as accurately by still earlier students. All the mycologists concerned in these redescriptions have been earnest strivers after truth, I am convinced, and would have preferred to employ the earlier names for their plants, could they have known that those earlier names referred with certainty to their specimens. All these people were relying, as was the usage of their time, on a few words of published description in some other than their mother tongue.

It is time to recognize generally that the resupinate *Hymenomycetes*, and especially the *Thelephoraceæ*, are extremely difficult taxonomic problems. Descriptions must include more than a rather vague and generalized characterization of the mere superficial appearance and habit of the specimen with possibly a reference to spores which some one recorded for what was perhaps this species. The fungus itself is an individual of the species; the description in words and by illustration has merit in proportion to the success it has in producing in the mind of any educated stranger exactly the ideas which he could derive from the study in detail of the specimen itself. From the specimen, exact ideas may be had of coloration, of form, of dimensions, of texture, of consistency, of internal structure, of organs of minute size, of place of growth, and of host and

substratum. If the description fails to give the color as exactly as if it had been noted by comparison with such a standard work as Ridgway's 'Color Standards' or Saccardo's 'Chromotaxia,' then it is inferior to the specimen; if the description contains no information as to whether the basidia are simple or cruciate, making up the whole hymenium or arranged side by side with other organs of characteristic form, standing directly on the substratum or separated from it by densely or loosely interwoven hyphæ or other form of subhymenial layer;—if it does not contain all this information in exact terms and as much in addition as the specimen itself could afford, then it is an imperfect description of the species. It may be so imperfect that a dozen different species of fungi could be assembled, to any one of which it would apply as well as to any other, as is the case with the supposedly common and cosmopolitan *Corticium lacteum* and *C. calceum*. Published exsiccati probably contain the full dozen under each of these names.

In the case of resupinate *Hymenomycetes*, types and authentic specimens of the species are of the highest importance to supplement the prevailingly imperfect descriptions with full and exact data. Hence, the types of fungi on which the descriptions are based and the authentic specimens from the authors of the species are of importance in proportion to the degree in which these plants may yield data not afforded by the descriptions and existing illustrations of the species. In the case of the resupinate *Hymenomycetes*, the early descriptions are of slight practical value except as they are backed up by types and specimens from their authors. For this reason, if there had been no other, the International Botanical Congress, at Brussels, acted for the best interests of mycology in fixing the beginning of the naming of *Hymenomycetes* with the publication of Fries' 'Systema Mycologicum,'—the time when the preservation of types and authentic specimens of such fungi in herbaria became so prevalent that it was possible for later mycologists to distinguish the resupinate species by taking the trouble to study the types, if authentic specimens could not be obtained.

My method of becoming acquainted with our described species of *Thelephoraceæ* has been to study and arrange by species in my herbarium the specimens as they have accumu-

lated. In this arrangement due regard has been given to original descriptions of species and to all details of internal structure. Spore collections on glass slides have been made for each species whenever possible, and about five thousand mounts of sectional preparations in glycerin have been made from collections and preserved for reference in connection with internal structure of the specimens. From time to time I have taken my *Thelephoraceæ* to herbaria where the types of our American species are stored and have there painstakingly matched them with the types. I have made sectional preparations from a fragment of each of these types in order to make sure that my specimens match the types not only in external characters but also in all details of internal structure. The sectional preparations of type specimens have been preserved in glycerin. Specimens from my herbarium which have been so matched with type specimens have been used by me later for the determinations of subsequent collections. Such methods of investigation are probably too laborious and require too much time to become popular and they afford little opportunity for the inspirational flights attributed to genius, but they do afford a means of determining within very narrow limits the species of North American *Thelephoraceæ*.

I am under especial obligation to Dr. W. G. Farlow for suggesting this work, for interest in its progress, and for frequent access to the Curtis Herbarium for comparisons with types. I am indebted also to Dr. C. H. Peck for opportunity to study his types in the New York State Herbarium, to the late Dr. L. M. Underwood for similar opportunity with the Ellis types in the Herbarium of the New York Botanical Garden, to Dr. S. W. Dixon and Professor S. Brown, of the Philadelphia Academy of Natural Sciences, for the privilege of studying in the Schweinitz Herbarium, to Sir W. T. Thistleton-Dyer and Mr. G. Massee for access to types and authentic specimens in Kew Herbarium, to the late Dr. T. M. Fries for the privilege of studying in the Herbarium of Elias Fries, at Upsala, and to Mr. Lars Romell, of Stockholm, Dr. P. A. Karsten, of Mustiala, and Abate G. Bresadola, of Trient, for many authentic specimens of their own species and for specimens which they had compared with types of early authors of *Thelephoraceæ* of

Europe. In the later pages names of the many botanists who have participated in this work by the contribution of specimens from their respective regions are given in connection with the specimens. I feel my obligation to each of these correspondents.

Having become thoroughly familiar with the species of a family of fungi, one then faces the task of deciding under what genera they shall be grouped in order that others may more easily recognize them. Our studies in systematic botany and the accumulations of plants in herbaria are primarily for the purpose of enabling those who wish to obtain information about any particular plant, however obscure, to determine its name accurately and so be in a position to get at the world's literature and knowledge concerning that species; and also to enable botanists so to entitle and index their researches that the results will be more available to the world at large. Stability in the nomenclature of plants is therefore important, and revolutionary changes in generic conceptions should not be lightly and frequently made. Whenever one proposes new genera to supersede a well-established genus which has satisfactorily embraced the related species of the world, the burden of proof should be on the one who makes the change to demonstrate that the advantages from the innovation will more than compensate for the confusion which would result as well as for the loss of knowledge indexed under the superseded name.

Many new genera of fungi have been proposed during recent years. These have frequently come from students with a limited knowledge of the species of the world. It is not surprising that a botanist working on the few species of a limited region should be led to the establishment of new genera on the basis of what seem to be sharp differences in his species or groups of species. When, however, his knowledge encompasses just as definitely the structure of the many species of some large portion of the world, his perspective changes, and he may now find that the species which he formerly regarded as generically distinct are so closely connected by intermediate species that the contemplated generic separation would be unnatural and a hindrance to botanical progress. It is fundamental that genera be so sharply defined that any accurate observer who will make

the study necessary for the application of the generic definition may be sure ninety-nine times out of a hundred that the fungus on which he is working is a *Stereum*, for example, and not a *Thelephora*, nor a *Craterellus*, nor a *Cladoderris*, nor a *Corticium*, nor a *Peniophora*, nor a *Sebacina*. It is an obligation on authors to group their species so accurately under genera that *Stereum*, for example, shall comprise all the species of this genus known to science, and no others. The synonomy of species in later pages will show how vaguely the genera of *Thelephoraceæ* have been comprehended.

It is desirable that a genus should consist of but few species in those cases where the group is sharply and naturally set off from others, that is, where no intermediate species connect the genus with other groups. While such small genera are desirable, if wholly natural, it is in the highest degree objectionable to create small artificial genera by arbitrarily segregating the species of a natural genus and so establishing indefinite lines of demarkation between genera. Under such a procedure the generic location of certain species becomes wholly arbitrary and always continues as a stumbling block for new students and this leads to the loading of our literature with so-called new species. A case in point is Saccardo's scheme in the 'Sylloge Fungorum' in which he separates *Hypochnus* from *Corticium* and *Peniophora* without any natural generic planes of cleavage. In practical work one needs to know exactly what the generic limits of *Corticium*, *Peniophora*, and *Hypochnus* are. The question naturally arises as to just how loose and open the structure of the fructification must be to be included in the genus *Hypochnus* rather than in *Corticium* or *Peniophora*. Henning's violation of the principle involved is still more flagrant, for he separated the *Hypochnaceæ* as a new family from the *Thelephoraceæ*[1] and placed *Hypochnus* of Saccardo in the *Hypochnaceæ*, and *Corticium* and *Peniophora* in the *Thelephoraceæ*. As all students of the *Thelephoraceæ* have found *Hypochnus*, as understood by Saccardo, wholly unworkable, it would increase the usefulness of the 'Sylloge Fungorum' if Saccardo were to distribute among *Corticium* and *Peniophora*, the species which he now includes under *Hypochnus*.

[1]Engler und Prantl, Nat. Pflanzenfam. (I. 1**): 114. 1898.

Probably all species of *Corticium*, as originally understood, have an hymenium composed of basidia arranged side by side between non-sporebearing organs termed paraphyses. In many species, it is difficult to distinguish between the basidia and the paraphyses except by prolonged study of special preparations or by observations made at the time the basidia bear spores. In other species the sterile organs are conspicuous and distinct from the basidia either by their larger size, different form, or thicker or incrusted walls. Such conspicuous bodies are called cystidia, but if the paraphyses are merely finely but characteristically branched near their tips, they are not called cystidia. Such branched paraphyses occur in the hymenium of occasional species of several genera of the *Thelephoraceæ* and are valuable characters for specific diagnosis.

In 1880, Cooke proposed, from Kew Herbarium, to divide the old genus *Corticium* into two genera,—the name *Corticium* to be retained for those species having the non-sporebearing organs of the hymenium not distinguishable from the basidia, and the generic name *Peniophora* to be given to those species having cystidia. As the species of *Corticium* were very numerous and extremely difficult taxonomically, this proposal was hopefully received, and for more than thirty years the transfer of species from *Corticium* to *Peniophora* has been going on and the end has not been reached yet. During this long period there has been confusion as to which species of the old genus *Corticium* belong in the emended *Corticium* and which in the genus *Peniophora*.

Peniophora is an artificial rather than a natural genus, however, and its adoption has given to many species a position intermediate between this genus and *Corticium*. These intermediate species have to be classed with the one genus or the other according to personal judgment, for no one can state just how conspicuous the sterile organs must be, nor of how constant occurrence, to merit the name cystidia. In *Corticium Sambuci* Fr., for example, cystidia are readily found in preparations from some collections, but several preparations may have to be made to demonstrate them in other collections. In the same species and in different parts of the same section, cystidia may sometimes be sparingly and sometimes not at all incrusted. Some

species which I have placed in the genus *Peniophora* because of the presence of cystidia students may look for under *Corticium* when, by a more hasty study of their collections, they fail to detect these organs. On the other hand, students using more discriminating methods than mine may detect cystidia in species in which I have overlooked them, and such students will search in *Peniophora* for species which I have placed under *Corticium*. Species intermediate between genera always cause such trouble. There are many intermediates between *Peniophora* and *Corticium*, yet in this particular case the advantage from the separation undoubtedly more than compensates for the disadvantages occasioned by the intermediate species.

The case of *Peniophora* has been considered at length, because this genus is being regarded as a precedent for subdividing *Stereum* and grouping under *Lloydella* all those species which have conspicuous non-sporebearing organs between the basidia. Such a separation, however, would be artificial and give rise to a troublesome series of intermediate species, without the compensating advantage which accrued in the case of *Peniophora* and *Corticium*. *Stereum* is not a genus of difficult species nor does it comprise an immense number of species. It is just a fine, natural group of species capable of being more sharply defined than it was by Fries, so as to receive some species from *Thelephora* of Fries and to part with some to *Corticium*. So defined, even beginners will have no trouble in recognizing species of *Stereum*. Systematic work in mycology should strive to establish and maintain just such natural, clean-cut genera as *Stereum*.

It seems to me best to work along constructive rather than destructive lines. Fries had a wonderful ability for the perception of the natural grouping of fungi on the basis of gross morphology and habit. Since his time, research has greatly enlarged the knowledge of the internal structure of fungi and of the organs of propagation. The value of such organs in the classification of seed plants is well known. It is feasible to modify somewhat the genera of *Thelephoraceæ* as defined by Fries, in accordance with the true relationships and differences shown by the present knowledge of internal structure, basidia, and spores, and a system results which is the natural evolution of taxonomic and morphologic study of *Thelephoraceæ*. This

system has been communicated to my correspondents in connection with specimens. Its principal features are:

1. To restrict *Thelephora* to pileate species with simple basidia and colored spores.
2. To follow Karsten and Bresadola in placing under *Hypochnus* only resupinate species with colored echinulate spores.
3. To restrict *Stereum* to pileate species which have simple basidia and colorless spores and lack setæ in the hymenium.
4. To include in *Hymenochæte* all species having setæ.
5. To include in *Corticium* species always resupinate, which have colorless spores and lack cystidia, excepting those species which for other reasons are placed in *Exobasidium*. Include in *Corticium* hypochnoid as well as compact species.
6. To include in *Peniophora* all species which differ from *Corticium* merely by the presence of cystidia.

I find this system workable and very satisfactory for the accurate location of species in genera, except in the case of the species intermediate between *Peniophora* and *Corticium*. The proposals to subdivide *Peniophora* into *Glœocystidium*, *Peniophorella*, *Glœopeniophora*, etc., would create large numbers of species intermediate between the new genera, without compensating advantages.

I have studied the species of my predecessors and co-workers sympathetically and have endeavored to find real differences between their species and those previously known so that the validity of theirs might be confirmed. The great area of land covered by the present work, the differences in climate and substratum, and the keen search by my correspondents have brought to hand a very large number of specimens. I have earnestly striven to place them under species already known, but it has been necessary to describe many as new. I regret that there are so many of these. Should any one have reason to believe that in any case I have described as new a species already known, I shall esteem it a favor to receive an authentic specimen of the older species or to be informed where such a specimen can be consulted.

Colors of specimens were noted and recorded during the first years of my work by comparison with Saccardo's 'Chromotaxia' in accordance with his descriptive terms. Recently I have been using Ridgway's 'Color Standards and Nomenclature,' 1912, which has a greater variety of colors useful in the characterization of the species of *Thelephoraceæ*.

In my own work with collections of living fungi I am endeavoring to gather for each species a spore collection on a glass slip. The spores adhere well so that they may be covered by paper and preserved in the envelope with the dried specimens from which the spores were obtained. Such collections give the exact color and dimensions of mature spores. These dimensions are generally rather larger than those obtained from spores of sectional preparations of dried herbarium specimens. The spores of dried specimens, i. e., those remaining attached to the specimens, are probably too immature to be of normal size, and sometimes there are so few of them that one must exercise caution to avoid errors due to the study of spores foreign to the fungus.

Latex exists in many species of several of the genera and is more abundant and conspicuous in some species than in others, and its containing elements often extend to the hymenial surface. When specimens are in the vegetative condition, injury to the hymenium may liberate the fluid contents of the latex bodies so that this fluid exudes in colored drops at the edges of the wound, or discolors the bruised surface. For many of our species there is a lack of data concerning the color of this fluid or the discoloration. The latex bodies are pale brown in microscopic preparations made by my methods and must not be confused with setæ or cystidia. Latex is well shown in *Stereum spadiceum*, *S. sanguinolentum*, and *Corticium lactescens*.

There has been a disposition on the part of some authors to regard the *Thelephoraceæ* as not sharply separated from the *Hyphomycetes*. The specimens which I have collected, in striving to find all the *Thelephoraceæ* of my collecting region, and the specimens received from my correspondents afford no embarrassment in recognizing the most hypochnoid species of *Thelephoraceæ* by the basidia which characterize the families of *Hymenomycetes* in general.

The microscopical technique has been simplified as much as possible. Usually dried herbarium material had to be used for study and proved very satisfactory except in the case of specimens which had been subjected to poisoning processes for preservation in herbaria. A small bit of the fructification having a promising hymenial surface 2 or 3mm. square—but smaller if the specimen is a valuable type—is first moistened with alcohol, then wet with water and cut out from the rest of the specimen and from the substratum. This bit is then placed in a holder of elder pith and oriented so that the sections may be cut perpendicular to the surface of the hymenium and also contain as long hyphæ as possible. The sections are cut as thin as possible, free hand, with a very keen section razor flooded with alcohol. The thinnest sections are placed on a slide in a drop of water and then a drop of seven per cent aqueous solution of potassium hydrate is added.

Close observation of the sections should be made when the potassium hydrate solution comes in contact with them. For most species, the sections are merely cleared and the hyphæ swelled to the normal size of vegetative hyphæ. In a few species, the alkaline solution may dissolve out the color of the section on coming in contact with it, or it may change this color to a violet, which finally disappears, or it may cause disorganization changes in certain structures leading to their disappearance or destruction. Such changes should be observed and noted, for they are of help in the determination of the species. In the cases in which potassium hydrate solution exerts a destructive action, lactic acid should be employed with other sections in the manner described for potassium hydrate. Lactic acid clears and swells sections well, but so much more slowly than potassium hydrate that I have used it only where the latter is not satisfactory. After the sections have been cleared, the potassium hydrate should be drained off, the sections lightly stained on the slide with alcoholic solution of eosin (but not overstained), mounted in water, and studied at once.

For a thorough study of the species of the family at least one permanent preparation of each species should be retained for future comparisons. Permanent preparations may be made from the temporary water mounts by adding dilute glycerin—

two-thirds glycerin and one-third water—at the edge of the cover glass and allowing the glycerin to run under the latter as the water evaporates. When concentration of the glycerin is adequate, the excess should be wiped away with moist filter paper and the resulting smear removed to the very edge of the cover glass with a soft cloth moistened with 95 per cent alcohol. The preparations may then be sealed from the atmosphere by painting a ring of microscopical cement about the edge of the cover glass. At least two coats should be used for this ring, a light and very narrow one, and, after this dries, a very heavy, broad one. I have used Bell's Microscopical Cement, made in London, and Brunswick Black Cement. A variable percentage of the rings crack in the course of a few years and allow the glycerin to escape from under the cover glass, but the sections in such preparations can be remounted. Dr. Thaxter has very recently informed me that he has been using King's Transparent White Cement and King's Amber Cement for fifteen years and that none of the rings made with these cements have cracked. By the use of circular cover glasses rather than square ones, a microscopist's turn table may be used, thereby materially lessening the labor of preparing the rings.

SYSTEMATIC ACCOUNT

THELEPHORACEAE

Thelephoreæ Persoon, Myc. Eur. 1: 109. 1822; Fries, Hym. Eur. 629. 1872; Saccardo, Syll. Fung. 6:513. 1888.

Hymenomycetes with the hymenium inferior or amphigenous (on the lower surface or surrounding the fructification), coriaceous or waxy, even, rarely ribbed or papillate.

Through several of the genera the *Thelephoraceæ* connect closely with all the other families of the *Hymenomycetes*. *Hypochnus* approaches *Grandinia* of the *Hydnaceæ* in the granular hymenial surface of many of the species, but can be separated from this hydnaceous genus by the spore characters. *Lachnocladium*, with coriaceous structure, hairy stem, and colorless spores, is an intermediate genus between *Clavaria*, of the *Clavariaceæ*, and *Thelephora* but can be separated from the latter by the spore characters. *Craterellus* connects with

Cantharellus, of the *Agaricaceæ*. Some species of *Corticium* must be cautiously separated from *Merulius*, of the *Polyporaceæ*. The species of *Tremellodendron*, *Hirneolina*, and *Sebacina* were formerly distributed among *Thelephora*, *Stereum*, and *Corticium* respectively, but are now separated from these genera by the cruciate character of the basidia,—such basidia as are present in many *Tremellaceæ*. All these connecting genera will be included in the present monograph.

Michenera and *Heterobasidium* are excluded genera. Lyman has shown[1] that *Michenera artocreas* B. & C. is only a stage in the life history of *Corticium subgiganteum* B. & C., and that the genus *Michenera* has ceased to be a genus of the Basidiomycetes. My own study of the type of *Heterobasidium chlorascens* Massee, which is the type species of the genus, failed to locate any basidia whatever.

Very many *Thelephoraceæ* are of great economic importance on account of the dry rot induced by the growth of the mycelium in sills, floors, mine, bridge, and dock timbers, and other wooden structures located in moist, poorly ventilated places. *Coniophora puteana* is a common species which rots coniferous wood. Only a very few *Thelephoraceæ* are classed as serious plant parasites. Of these the rhizoctonial stage of *Corticium vagum* is the most important.

Key to the Genera

I. EU-THELEPHOREÆ:

Fructification not containing green lichen gonidia.

Fructification fleshy or membranaceous, often infundibuliform, with the hymenium distinct, continuous, even, ribbed or at length rugose; basidia simple ..... *Craterellus*

Fructification submembranaceous, cup-shaped, often pendulous; hymenium typically concave, discoid; basidia simple ..... *Cyphella*

Fructification consisting of only a fleshy hymenium on the surface of living leaves and shoots; basidia simple ..... *Exobasidium*

Fructification coriaceous or hard 1

1. Basidia globose or pyriform, longitudinally cruciately 4-septate or divided when mature; fructification erect, clavariform, more or less branched ..... *Tremellodendron*

[1] Cultural studies on the polymorphism of Hymenomycetes. Proc. Boston Soc. Nat. Hist. 33: 151-60. 1907.

1. Basidia cruciate as in *Tremellodendron;* fructification effuso-reflexed or cup-shaped with the margin free ..... *Hirneolina*
1. Basidia cruciate as in *Tremellodendron;* fructification always resupinate. *Sebacina*
1. Basidia simple but with such large sterigmata as to resemble longitudinally divided basidia[1] ..... *Tulasnella*
1. Basidia at first globose and simple, at length elongated and transversely septate, straight or curved, bearing sterigmata on the convex side; fructification resupinate ..... *Septobasidium*
1. Basidia simple, usually 4-spored 2
   2. Spores colored; fructification pileate ..... *Thelephora*
   2. Spores colored, rough-walled to echinulate; fructification resupinate ..... *Hypochnus*
   2. Spores ochraceous, ferruginous or fuscous, even; fructification resupinate .. *Coniophora*
   2. Spores white or rarely bright colored, even or rarely uneven 3
3. Setæ (brown, cylindric, rigid, even-walled bodies) present in the hymenium; fructifications range from pileate to resupinate ..... *Hymenochæte*
3. Cylindric teeth composed of many consolidated hyphæ protrude from the hymenium but are not covered by it. Our southern species was originally described as a *Hydnum* ..... *Mycobonia*
3. Neither setæ nor teeth present in the hymenium 4
   4. Fructification coriaceous, erect, clavariform; stem often hairy .. *Lachnocladium*
   4. Fructification cup-shaped, resupinate with free margin or simply resupinate; hymenium pulverulent; with some two or three of the following characters: (1) large white spores ranging from 14–34 x 12–20 μ; (2) much granular matter in the fructification; (3) prominent moniliform or branched paraphyses; (4) racemose organs in the hymenium which produce a crop of conidia before basidiospores develop ..... *Aleurodiscus*
   4. Fructification pileate ranging from infundibuliform and flabelliform to very narrowly reflexed forms; hymenium even. Some reflexed species may occur resupinate ..... *Stereum*
   4. Fructification like that of an urn-shaped *Stereum* but hard and stuffed. One tropical species ..... *Hypolyssus*
   4. Fructification like that of *Stereum* but with the hymenium hardened and with radiating branched ribs. Species tropical ..... *Cladoderris*
   4. Fructification always resupinate; structure not as in *Aleurodiscus* 5
5. Subhymenial tissue contains conspicuous brown stellate organs composed of several radiating arms ..... *Asterostroma*
5. Such brown stellate organs not present 6
   6. Cystidia present in hymenium, or in subhymenial tissue, or in both; structure may be compact or hypochnoid ..... *Peniophora*
   6. Cystidia not present; structure compact or hypochnoid ..... *Corticium*

[1] With regard to the nature of these bodies see H. O. Juel, Bihang till K. Sv. Vet.-Akad. Handl. 23[II]: Afd. III. 3–27. 1897.

II. HYMENO-LICHENS:

Fructification regularly containing green lichen gonidia. Species tropical.

Fructification pileate, coriaceous-membranaceous, with hymenium on the lower surface and somewhat waxy; gonidial layer composed of somewhat cubical masses of algal cells ..... *Cora*

Fructification like *Cora* in most respects but with the hymenium somewhat gelatinous and the gonidial layer composed of algal cells arranged in rows (cateniform) ..... *Rhipidonema*

THELEPHORA

Thelephora Ehrhart [Crypt. Exsic. No. 178. 1785] Fries, Syst. Myc. 1: 428. 1821 (in part).–Persoon, Myc. Eur. 1: 110. 1822 (in part).–Saccardo, Syll. Fung. 6: 521. 1888 (in part).–Hennings, in Engl. &. Prantl, Nat. Pflanzenfam. (1. 1**): 125. 1898 (in part).

The type species of the genus is *Thelephora terrestris* Ehrh. ex Fries.

Fructifications pileate or clavate, coriaceous; hymenium continuous with the hymenophore and similar to it, inferior, or amphigenous in a few species, even or faintly ribbed or papillose; basidia simple, 4-spored; spores colored, typically muricate but even, or rough-walled in a few species.

As more broadly defined by Fries and the other authors cited, *Thelephora* has been heterogeneous, consisting chiefly of the natural and homogeneous group of species defined above but also of some pileate species with simple basidia and hyaline spores, transferred to *Stereum;* also of some species with globose, longitudinally septate basidia, transferred to *Tremellodendron*, if with erect fructifications, or to *Sebacina*, if resupinate; and also of some resupinate species having simple basidia, of which those with muricate and colored spores may be found in *Hypochnus*, those with colored and even spores, in *Coniophora*, and those with hyaline spores, in *Corticium* and *Peniophora*. It is probable that the species of Patouillard's section *Dendrocladium* of the genus *Lachnocladium* as understood by Patouillard[1] might be transferred to *Thelephora* with advantage both to *Thelephora* and *Lachnocladium*, but these species are not within the geographical limits of my work.

[1] Fragments Mycologiques (suite). Jour. de Bot. 3:33–37. 1889.

KEY TO THE SPECIES

Erect species, usually with central stem and pileus divided into very narrow, branching, flattened or cylindric divisions; hymenium inferior or amphigenous 1

Erect species, usually with central stem and more or less infundibuliform, cup-shaped or flabelliform pileus, which may be radially split into lobes and divisions 2

Species of incrusting, effuso-reflexed, dimidiate, or applanate habit 5

1. 2–6 cm. high, much branched, glabrous, with fetid odor when growing, perhaps rarely odorless ..... *1. T. palmata*

1. 3–5 cm. high, much branched, minutely pubescent; stem villose, without fetid odor. Compare *T. multipartita* ..... *2. T. anthocephala*

1. Less than 2½ cm. high, branching at or below surface of ground, dusky drab except at base ..... *3. T. cæspitulans*

1. Less than 2 cm. high, very slender and fragile, cinereous. Known only from State of Washington ..... *4. T. scissilis*

1. Large species, highly branched, with body of spore of regular obovoid form. Known only from Central America ..... *5. T. angustata*

2. Hymenium dark colored, i. e., brown to fuscous 3

2. Hymenium light colored, i. e., pallid to gray 4

3. Small species, 1½–3 cm. high, upper surface usually drying pallid, usually deeply cleft or many-parted into narrow divisions; stem villose. *6. T. multipartita*

3. Small species, 6 mm.–2½ cm. high, infundibuliform or deeply divided into two or three triangular divisions, or flabelliform; stem villose. Closely related to *T. multipartita* ..... *7. T. regularis*

3. Fructification 1 cm. high, white; stem white, glabrous. Known only from Guadaloupe ..... *8. T. pusiola*

3. 1½–5 cm. high, larger species than the three preceding but with thinner pileus, fuscous purple (Rood's brown) throughout, often with the thin lobes imbricate like the petals of a carnation; stem villose ..... *9. T. caryophyllea*

3. 2–4 cm. high, somewhat tubular, hymenium vinaceous brown to drab; stem sulcate and pitted but not villose; spores 10–14 μ in diameter. Known only from Jamaica ..... *10. T. magnispora*

3. Large species, 2½–7 cm. in diameter, with upper surface pallid except at the center and with the hymenium dark ..... *13. T. vialis*

4. Small species, less than 2 cm. in height and in diameter, somewhat pallid to brick-red ..... *7. T. regularis*

4. Pileus with outer lobes forming a cup and with inner lobes distinct, crowded, erect, cinereo-fuscous. Known from Costa Rica and Brazil. *11. T. corbiformis*

4. Large species, 5–7 cm. broad, deeply infundibuliform, habit and color of *Craterellus cornucopioides*. Costa Rica and Jamaica. *12. T. cornucopioides*

5. Growing in applanate clusters, effuso-reflexed, or dimidiate 6

5. Always incrusting (*T. albido-brunnea* is sometimes incrusting) 8

6. Hymenium pale and colored like the pileus, cinnamon-buff; pileus spongy, more than 2 mm. thick; spores 8–10 x 6–8 μ ... *14. T. albido-brunnea*

6. Hymenium and pileus yellowish, less than 2 mm. thick; spores 5–6 x 4 μ *15. T. lutosa*

6. Hymenium drab, becoming sage-green when crushed in 7 per cent potassium hydrate solution; pileus pinkish buff to cinnamon-brown with a broad pale margin ..... *16. T. cuticularis*

6. Hymenium ferruginous brown (Rood's brown) to fuscous 7

7. Pileus, when squamulose, with the fibers matted and agglutinated into appressed and wholly adnate squamules, margin dilated and whitish fimbriate becoming entire and concolorous ..... *17. T. intybacea*

7. Pileus not zonate, fibrous-squamulose and usually strigose, margin fibrous-fimbriate ..... *18. T. terrestris*

7. Pileus zonate, in other respects resembling the preceding species ..... *19. T. griseozonata*

8. Incrusting and ascending small plants, free branches somewhat terete but flattened towards the tips; spores umbrinous ..... *20. T. fimbriata*

8. Resupinate on leaves and twigs on the ground and sending up free, simple or branching trunks; spores fuscous. Known from Cuba only *21. T. perplexa*

8. Incrusting leaves, etc., on the ground and ascending as sessile flabelliform pilei which are dentate at the upper end or deeply divided, honey-yellow to tawny olivaceous throughout. Known from Cuba only .... *22. T. dentosa*

8. Typically effused, rising obliquely upward from the support as a cluster of small trunks which branch and terminate in spiculous tips. *23. T. spiculosa*

1. **Thelephora palmata** Scop. ex Fries, Syst. Myc. 1: 432. 1821. Plate 4. fig. 4.

Clavaria palmata Scop. Fl. Carn. 2: 483. 1760.–*Ramaria palmata* Holmsk. Fun. Dan. 1: 106. *pl.*—.1799. –*Merisma foetidum* Pers. Syn. Fung. 584. 1801.–*M. palmata* Pers. Myc. Eur. 1:113. 1822.–*Thelephora palmata americana* Peck, Rep. N. Y. State Mus. 53: 857. 1900.

Illustrations: Greville, Crypt. Fl. 1: *pl. 46.*–Holmskiold, Fun. Dan. 1: *pl.* of *Ramaria palmata.*–Krombholz, Abbild. und Beschr. *pl. 54. f. 24, 25.*–Nees, System *pl. 16. f. 151 B.*–Baillon, Dictionn. de Botan. 1: 737. *f. 7.*–Loudon, Encyc. of Plants *f. 16131.*–Winter, Crypt. Flora 1: 321.

Fructification coriaceous-soft, fuscous purple, drying cinnabar-brown or chestnut-brown, erect, very much branched, with very fetid odor; pileus with numerous somewhat fastigiate, palmate divisions which are even, flattened, dilated above, and with fimbriate and whitish tips; stem simple or soon branched; hymenium amphigenous; spores pale umbrinous under the microscope, sparingly echinulate, 10 x 7–8 μ.

Fructification of American specimens 2–6 cm. high, 1–3 cm. broad; stem 1–1½ cm. long, 1–2 mm. thick.

On moist ground in coniferous woods and also in grassy fields. Prince Edward Island to North Carolina and west to Illinois. June to October.

In the American collections of this species the divisions of the pileus are narrow and a short stem is present. The habit is so similar to that of *Thelephora anthocephala* that record of the fetid odor should always be made if observed. The ultimate branches may be more or less terete, leading to the variety *americana* Pk.

Specimens examined:

Exsiccati: Ell. & Ev., N. Am. Fungi, 1937.

Austria: *G. Bresadola.*[1]

Sweden: *L. Romell, 53.*

Canada: Rustico Bay, Prince Edward Island, *J. Macoun, 324.*

New Hampshire: Chocorua, *W. G. Farlow.*

? Vermont: no locality data for specimen in Frost Herb., Univ. of Vermont.

Connecticut: Manchester, *C. C. Hanmer, 1398.*

New York: Fischer's Island, *C. C. Hanmer, 196.*

New Jersey: *C. G. Lloyd, 4612.*

Pennsylvania: Bethlehem, *Schweinitz,* Syn. N. Am. Fungi, 612 (in Herb. Schw.); Trexlertown, *Dr. W. Herbst;* Kitanning, *D. R. Sumstine, 2;* West Chester, *B. M. Everhart,* Ell. & Ev., N. Am. Fungi, 1937.

Delaware: Newark, *H. S. Jackson.*

Dist. of Columbia: Washington, *O. F. Cook,* comm. by P. L. Ricker, 1, 3.

N. Carolina: Asheville, *H. C. Beardslee, 924.*

Ohio: Connecticut, *C. G. Lloyd, 4493.*

Illinois: Glencoe, *E. T. and S. A. Harper, 664, 665.*

Missouri: St. Louis, *N. M. Glatfelter* (in Mo. Bot. Gard. Herb., 42560).

[1] With regard to the citation of specimens all except those of "Exsiccati" are in Burt Herb. which are cited without explicit reference to place in other herbaria. For example, the specimen cited, "Connecticut: Manchester, *C. C. Hanmer, 196,*" is in Burt Herb. The data given is that received with the specimen and may identify a duplicate in another herbarium. The location of all specimens in herbaria other than my own is designated by the name of the herbarium in parenthesis with the prefix "in." For example, the specimen cited, "Louisiana: St. Martinville, *A. B. Langlois* (in Lloyd Herb., 3000)," is in Lloyd Herb., but not in Burt Herb.

2. **T. anthocephala** Bull. ex Fries, Syst. Myc. 1: 433. 1821. Plate 4. fig. 1.

Clavaria anthocephala Bull. Herb. de la France 2: 197. *pl. 452. f. 1.* 1789.

Illustrations: Bulliard, *Ibid. pl. 452. f. 1.*–Sowerby, Col. Figs. Eng. Fun. *pl. 156.*–Berkeley, Outlines Brit. Fung. *pl. 17. f. 4.*–Dufour, Atlas des Champ. *pl. 70.*

Fructification coriaceous-soft, somewhat ferruginous, drying fawn-color or cinnamon-brown, inodorous; pileus pubescent, divided to the stem into flaps which are dilated upwards and fimbriate and whitish at the apex or divided into irregular, branched, erect branches; stem simple, equal, villose; hymenium even; spores pale umbrinous under the microscope, ranging from angular-tuberculate to tuberculate-echinulate, 8–10 x 7–8μ.

Fructifications 3–5 cm. high, 1–3 cm. broad; stem 1–1½ cm. long, 1–2 mm. thick.

On the ground in woods. Massachusetts and Ohio to Louisiana. June to August. Rare.

Our specimens of *T. anthocephala* and *T. palmata* have the same habit but may be separated, even when dried, by the fine pubescence of the pileus visible with a lens, and by the villose-tomentose stem of the former. The spores of *T. anthocephala* are further slightly paler and have shorter spines with broader bases than those of *T. palmata.*

Specimens examined:

Austria: *G. Bresadola.*

Massachusetts: Newton, *W. G. Farlow* (in Farlow Herb.).

New York: Van Cortlandt Park, N. Y. City, *L. O. Overholts* (in Overholts Herb., 688).

Pennsylvania: Kitanning, *D. R. Sumstine, 10;* Bethlehem, *Schweinitz* (in Herb. Schw.), the 614 of Syn. N. A. Fungi under the name *T. flabellaris.*

North Carolina: Asheville, *H. C. Beardslee, 0268.*

Louisiana: St. Martinville, *A. B. Langlois, unnumbered specimen,* and *1971,* and by the same collector (in Lloyd Herb., 3000).

Ohio: Norwood and Linwood, *C. G. Lloyd, 1472* and *02164* respectively.

Kentucky: *C. G. Lloyd, 1395.*

Missouri: St. Louis, *N. M. Glatfelter* (in Mo. Bot. Gard. Herb., 42559).

3. T. caespitulans Schw. Trans. Am. Phil. Soc. N. S. 4: 166. 1831.[1]

Type: in Herb. Schweinitz.

Fructification erect, coriaceous, dusky drab to olive-brown below, paler above, very much branched, forming clusters 2½ cm. high by 2½ cm. broad; pileus with numerous divisions joined together into a solid base but assurgent above and pressed together closely, compressed, subcanaliculate, frequently obtuse and whitish at the apex; hymenium amphigenous; spores umbrinous under the microscope, sparingly tuberculate, 7–8 x 5–6μ.

On the ground in mixed woods, Vermont to South Carolina, and in dense coniferous woods, Washington. September. Rare.

This species is related to *T. palmata* but is more olivaceous, and it is probably inodorous,—at least no odor has been noted. The dimensions for the clusters given above, as stated by Schweinitz, are probably maximum dimensions, for the specimens recently collected have been rather smaller. My Vermont specimens were growing with the thick, solid base buried in sandy ground in a wood road; they have dried pallid except at the base and are slightly pubescent. The general habit of this species is somewhat suggested by a small cluster of *Tremellodendron pallidum* (Schw.) Atk.

Specimens examined:

Vermont: Lake Dunmore, *E. A. Burt.*

Pennsylvania: Bethlehem, *Schweinitz,* type (in Herb. Schw., Acad. Nat. Sci., Phila.).

South Carolina: Santee Canal, *Ravenel, 1660* (in Curtis Herb. under name *T. vialis*).

Washington: Chebalis, *C. J. Humphrey, 1287;* Bingen, *W. N. Suksdorf, 689.*

4. T. scissilis Burt, n. sp. Plate 4. fig. 8.

Type: in Burt Herb.

Fructifications gregarious, coriaceous, erect, clavariform, branched, longitudinally ridged by the bases of numerous,

[1] A figure will be given in Part II.

small, appressed, acicular branches, the larger of which are at the apex of the fructification and spread slightly outward in fan-shaped manner; stem glabrous, castaneous; hymenium amphigenous, on upper half of the fructification, avellaneo-cinereous; basidia simple, hyaline, 4-spored; spores pale umbrinous under the microscope, angular, 6–8 x 5–6μ.

Fructifications 1½–2 cm. high; spread of branches at the top 2-6 mm.; stem 7–10 mm. long, 1 mm. thick.

On the ground. Washington. January.

This species is very distinct by its slender erect habit, cinereous color, and only slightly spreading branches.

Specimens examined:

Washington: Bingen, Klickitat Co., *W. N. Suksdorf, 716*, type.

5. **T. angustata** Fries, (Nov. Symb. Myc. 92.) Actis R. Soc. Sc. Upsal. III. 1: 108. 1851.

Type: in Herb. Fries.

Fructification erect, cinereo-fuscous, pliant, becoming rigid and somewhat woody; stem elongated, radicated, rugose, glabrous, compressed, irregularly divided at the upper end into unequal, fastigiate, compressed branches, which are clothed on the whole outer surface with the hymenium; hymenium amphigenous, subrugose, gray; basidia simple; spores umbrinous under the microscope, obovoid, apiculate at base, flattened on one side, echinulate, 14 x 7–9μ.

On decaying wood. Central America.

Substance, color, and hymenium exactly as in *T. cornucopioides*, but of the very different form of *Clavaria rugosa* and having highly branched forms; stem 5 cm. long; color fuliginous. The fructification is fleshy-pliant when fresh, but on drying hardens much more than species of *Stereum*.

Specimens examined:

Costa Rica: *Oersted* (in Herb. Fries), type.

6. **T. multipartita** Schw. in Fries, Elenchus Fung. 1: 166. 1828. Plate 4. fig. 7a.

Type: in Herb. Schweinitz.

Fructifications gregarious, erect, coriaceous, fusco-cinereous, usually drying pallid; pileus infundibuliform, sometimes cleft

more or less deeply and unequally into a few lobes, sometimes divided to the stem and spreading so as to appear dimidiate, very often deeply divided and subdivided into many narrow and spreading divisions more or less dilated and whitish at the apex; stem erect or incurved, equal or tapering upward, sometimes branched above, drying walnut-brown or pallid, villose; hymenium inferior, glabrous, even, fawn-color or vinaceous drab; spores unbrinous under the microscope, tuberculate, 7–9 x 5–6μ.

Fructification 1½–3½ cm. high, 1–3 cm. broad; stem 1–2 cm. long, 1–3 mm. thick.

On ground in groves of broad-leaved trees, especially under oak. New York and Pennsylvania to Illinois. July to September.

The upper surface of the pileus was originally described as glabrous, but it is minutely pubescent under a lens, or sometimes fibrillose. This species is very perplexing by its close relationship to *T. regularis*. The multipartite pileus is the only character which seems available to separate collections of the former from the latter species. If a given collection consists wholly of specimens with pileus many-parted and subdivided into narrow divisions, or if it contains some such specimens in addition to others with more regular infundibuliform pileus, I refer the collection to *T. multipartita*, as in the cases of the collections cited below from C. O. Smith and Dr. C. H. Peck respectively. As yet, I know of no characters by which to assort and separate into their respective species specimens mixed together of typical *T. regularis* and those specimens of *T. multipartita* which have the pileus infundibuliform or merely cleft more or less deeply and unequally into a few lobes. Therefore it is my opinion that *T. multipartita* is a variety of *T. regularis*, but the collections which have so far been submitted to me, have been composed of too few fructifications to assure me that this opinion is correct.

Specimens examined:

Exsiccati: Ell. & Ev., N. Am. Fungi, 2806, under the name *T. caryophyllea*.

New York: Bolton, *C. H. Peck, 3, 4, 5;* Ithaca, *C. O. Smith,* Cornell Univ. Herb., 13359, and *C. O. Smith and W. H. Long,* Cornell Univ. Herb., 7743.

New Jersey: Newfield, *J. B. Ellis,* Ell. & Ev., N. Am. Fungi, 2806.

Pennsylvania: on island in Lehigh River, *Schweinitz,* type (in Herb. Schw.); Bethlehem, *Schweinitz* (in Herb. Schw.), the *T. tuberosa* of Syn. N. Am. Fungi, 613; Trexlertown, *W. Herbst, 22, 36.*

Ohio: *A. P. Morgan,* Lloyd Herb., 2581, 2647; Oxford, *L. O. Overholts* (in Overholts Herb., 1685).

Illinois: River Forest, *E. T. and S. A. Harper, 666.*

7. **T. regularis** Schw. Schrift. d. Naturforsch. Gesell., Leipzig, 1: 105. 1822. Plate 4. figs. 6, 7b.

Thelephora Ravenelii Berk. Grevillea 1: 148. 1873.–*T. hiscens* Berk. & Rav. Grevillea 1: 148. 1873.

Type: in Herb. Schweinitz, Acad. Nat. Sci., Phila.

Pileus coriaceous, solitary, infundibuliform or divided to the stem into triangular divisions or flabelliform, fibrillose, drying pallid or tawny-olive, darker at center of the cup or at base of the divisions, margin lacerate; hymenium usually hair-brown, sometimes pallid; spores melleus to umbrinous under the microscope, angular-tuberculate, 6–7 x 4½–5μ.

Fructification 6 mm.–2½ cm. high; pileus 5 mm.–2½ cm. broad; stem 3–15 mm. long, 1–1½ mm. thick.

In moss in wet places and on humus. Ontario to Alabama and westward to Kansas.

The differences in form of the pileus of *T. regularis* are well shown by the type in Herb. Schweinitz; this type consists of three fructifications, two of which are infundibuliform, the third and largest, flabelliform. The hymenium is sometimes merely pallid, as in the case of the specimen which is the *T. pannosa* of Schweinitz, Syn. N. Am. Fungi, No. 606, but is not *T. pannosa* Fr. The cotypes of *T. Ravenelii* and *T. hiscens* agree in all respects with the authentic specimen of *T. regularis* in Curtis Herb. Specimens of *T. regularis* which have the pileus infundibuliform and little cleft are suggestive of small specimens of *T. caryophyllea* but differ from the latter by the thicker pileus

and paler coloration of *T. multipartita* which is wholly lacking in the rufescent coloration of *T. caryophyllea*. There is a difference of form between specimens of these two species which is brought out well by the figures in pl. 4.

Specimens examined:

Canada: Shannonville, Ontario, *J. Macoun, 330.*

Maine: Portage, *L. W. Riddle, 4.*

New Hampshire: Chocorua, *W. G. Farlow* (in Farlow Herb.).

Massachusetts: near Boston, *Sprague, 246* (in Curtis Herb. under the name *T. anthocephala*); Newton, *W. G. Farlow* (in Farlow Herb.).

Pennsylvania: Bethlehem, *Schweinitz,* station cited by Schweinitz; also the specimen (in Herb. Schw.) under the name *T. pannosa* of Syn. N. Am. Fungi, No. 606; Trexlertown, *C. G. Lloyd;* Kitanning, *D. R. Sumstine.*

Delaware: Clayton, *H. S. Jackson.*

North Carolina: Salem, *Schweinitz,* type (in Herb. Schw.); *G. F. Atkinson,* Cornell Univ. Herb., 23254.

South Carolina: Greenville, *Ravenel, 1498,* type and cotype (in Kew Herb. and in Curtis Herb. respectively) of *T. Ravenelii* Berk.; Santee Canal, *Ravenel,* type and cotype (in Kew Herb. and in Curtis Herb. respectively) of *T. hiscens* Berk. & Rav.

Alabama: *Peters, 576 bis* (in Curtis Herb. under the name *T. anthocephala*).

Wisconsin: Madison, *W. Trelease* (in Farlow Herb.); Lake Geneva, *E. T. and S. A. Harper, 882,* and (in Harper Herb., 883).

Illinois: East St. Louis, *N. M. Glatfelter* (in Mo. Bot. Gard. Herb., 42563).

Iowa: Johnson County, *T. J. Fitzpatrick, 39.*

Missouri: St. Louis, *N. M. Glatfelter* (in Mo. Bot. Gard. Herb., 42564).

Kansas: Bourbon County, *A. O. Garrett, 80.*

8. T. pusiola Pat. in Duss, Champ. Guad. & Martinique 12. 1903.

Pileus with divisions triangular, white, hard, thin, entire or cut-lobed, glabrous, even or rugose, sometimes zonate, atten-

uated into a slender stem; stem colored like the pileus, glabrous, cylindric, woody; hymenium inferior, even, brown; basidia clavate, 25 x 10μ, four-spored; spores globose-angular, colorless or somewhat fuliginous, 6 μ in diameter; no cystidia.

Fructification 1 cm. high, divisions 5 mm. broad.

Solitary or in clusters on dead wood. Guadaloupe. Forest of Bains-Jaune, *Duss, 589.*

Var. *terrestris* Pat. *Ibid,* has the divisions of the pileus narrower, laciniate, divergent, rigid.

On the ground, Matouba, Guadaloupe, *Duss.*

I have seen no specimens of either this species or its variety, neither of which have been reported since their original discovery.

9. **T. caryophyllea** Schaeffer ex Fries, Syst. Myc. 1: 430. 1821. Plate 4. fig. 9.

Elvella caryophyllea Schaeffer, Icon. Fung. 3: 115. *pl. 325.* 1762–1774.–*Craterella ambigua* Pers. Obs. Myc. 1: 36. *pl. 6. f. 8–10.* 1796.–*Thelephora caryophyllea* γ *ambigua* Pers. Myc. Eur. 1: 112. 1822.

Illustrations: Schaeffer, Icon. Fung. *pl. 325.*–Persoon, Obs. Myc. 1: *pl. 6. f. 8–10.*–Schnizlein, in Sturm, Deutsch. Flora 3: fasc. 31. *pl. 6.*–Lanzi, Fungi di Roma *pl. 11. f. 4.*–Saunders and Smith, Myc. Ill. *pl. 41. f. 7–12.*–Smith, W. G. Brit. Basid. 399. *f. 96 a, b.*

Fructifications solitary or cespitose, coriaceous, fuscous purple but drying wood-brown; pileus infundibuliform, simple, or doubled by proliferous growth of smaller pilei from the disk of the principal pileus or of wedge-shaped lobes rising from its upper surface, upper surface radiately ridged or striate with masses of agglutinated fibers which are often dark colored, obscurely zonate when moist, margin incised; stem usually central, cylindric, villose, simple or branched; hymenium inferior, even, grayish olive to light yellowish olive; spores pale umbrinous, tuberculate, 7–8 x 6μ.

Fructification 1½–5 cm. high, 1½–5 cm. broad; stem 1 cm. long, 2–3 mm. thick.

On the ground under pines. Canada to South Carolina and west to Ohio, also in the Pacific states. August to November. Abundant locally.

T. caryophyllea may be distinguished from our other northern species which have a central stem and dark hymenium, by the thin lobes of the pileus which dry paler than the hymenium, and by the frequent occurrence of specimens with the pileus consisting of many lobes and pilei imbricately arranged in a manner suggestive of a double pink or carnation, as shown by Schaeffer's fig. 5, and Persoon's fig. 10 of the illustrations cited. Our specimens agree well with the figures of Schaeffer and Persoon—those of Persoon are especially good but unfortunately occur in a work which is very rare.

We find occasionally specimens which agree well with *T. radiata* (Holmsk.) Fr., but these specimens are connected so closely by intermediate forms—often in the same collection—with others which are undoubtedly *T. caryophyllea* that I refer them to the latter species.

Specimens examined:

Sweden: *K. Starback*, in Romell, Fun. Scand., 121.

Canada: *J. Macoun, 54* and *75* of 1903.

Quebec: Hull, *J. Macoun, 190.*

Ontario: London, *J. Dearness* (in Lloyd Herb.).

New Brunswick: Restigouche River, *T. F. Allen*, comm. by Dr. Farlow.

Maine: Orono, *L. W. Riddle, 9.*

New Hampshire: Shelburne, *W. G. Farlow.*

Vermont: Newfane, *C. D. Howe;* Middlebury, *E. A. Burt*, four collections.

Massachusetts: *Sprague, 47, Russell, 131*, and *D. Murray, 545* (all in Curtis Herb.); Worcester, *G. E. Francis, 105.*

Connecticut: East Hartford, *C. C. Hanmer, 1449;* Central Village, *J. L. Sheldon, 68*, comm. by New York Bot. Gard.

New York: Bolton, *C. H. Peck;* Ithaca, *G. F. Atkinson, 9993, 9994;* Saranac Lake, *E. A. Burt;* East Galway, *E. A. Burt.*

Pennsylvania: Bethlehem, *Schweinitz* (in Herb. Schw.), the 608 of Syn. N. Am. Fungi.

Dist. of Columbia: Zoölogical Park, *Coville and Cook, No. A*, comm. by P. L. Ricker.

North Carolina: *Schweinitz* (in Herb. Schw.).

Michigan: *C. G. Lloyd, 4547;* Sailor's Encampment, *E. T. and S. A. Harper, 439,* and Univ. of Wis. Herb., 2.

Ohio: *C. G. Lloyd, 1422, 2720;* Cincinnati, *A. P. Morgan,* Lloyd Herb., 2641, and (in Lloyd Herb., 1152); Loveland, *D. L. James* (in Herb. U. S. Dept. Ag.).

Kentucky: *C. G. Lloyd, 1152.*

Washington: Bingen, *W. N. Suksdorf, 717, 690.*

California: Jackson, *J. H. Barber,* comm. by W. A. Setchell; Stanford University, *C. F. Baker, 255,* distributed by Baker, Pacific Slope Fungi, 3743, under the name *T. radiata* (Holmsk.) Fr.

10. **T. magnispora** Burt, n. sp. Plate 4. fig. 5.

Type: in Burt Herb.

Fructifications solitary or gregarious, coriaceous, stipitate; pileus irregularly infundibuliform, somewhat tubular, with ascending recurved lobes, drying avellaneous, becoming fuscous at the center with age, fibrous torn becoming radiately striate, margin incised; stem equal, solid, drying hard, irregularly angled, sulcate and pitted, vinaceous brown to drab; hymenium inferior, even, vinaceous brown; basidia simple; spores pale cinnamon, subglobose, echinulate, 10–14 μ in diameter.

Fructification 2–4 cm. high; pileus 1–2 cm. in diameter; stem 7–12 mm. long, 2–5 mm. thick.

On mossy ground. Chester Vale, Jamaica. December.

In some of the specimens the pileus is decidedly eccentric through greater growth on one side than on the other, and it is not always lobed. The offensive odor of the dried specimens and the color of the hymenium are suggestive of *T. cuticularis.*

Specimens examined:

Jamaica: Chester Vale, *W. A. and Edna L. Murrill,* type, New York Bot. Gard., Fungi of Jamaica, 295.

11. **T. corbiformis** Fries, (Nov. Symb. Myc. 92.) Actis. R. Soc. Sc. Upsal. III. 1: 108. 1851.–Romell, Hymenomycetes Austro-Americani. Bihang till K. Sv. Vet.-Akad. Handl. 26[16]: Afd. III. 44. 1901.

Type: in Herb. Fries.

Fructification sessile, rigid, cinereo-fuscous, with cespitose lobes of which the outer ascend and coalesce into a rounded

cupulate pileus here and there lacunose-pervious, and the inner are distinct, crowded, erect, narrow; hymenium inferior, uneven, whitish; basidia simple; spores slightly colored, becoming uneven, ovoid, 5–6 x 4–5 μ.

On the ground. Costa Rica and Brazil. January.

"In substance, texture, color, etc., this species agrees exactly with *Thel. cornucopioides* and *Thel. angustata* but in form it exhibits a type unique in the Hymenomycetes. The clusters are regularly rounded, very dense, divided all the way to the base into innumerable lobes, of which the interior are free and erect, the exterior regularly ascendant, broader, compressed, clothed underneath by the hymenium and grown together into a cup here and there lacunose-pervious, undulate-crisped at the apex and fimbriate."—Translation of the original comment on this species.

In 1899, I found the type in Herb. Fries to be cinereo-pallid with a slight fuscous tinge and with basidia and spores as stated above but many of the spores even. Romell describes the spores of his specimens from Brazil as "hyalinæ, laeves, ellips., 5–7 x 3–4 mmm.," and as agreeing with the type. I have reëxamined my sections from the type; the spores are certainly colored and many of them rough-walled.

Specimens examined:

Costa Rica: San José, *Oersted* (in Herb. Fries, Univ. Upsal.), type.

12. **T. cornucopioides** Fries, (Nov. Symb. Myc. 91.) Actis R. Soc. Sc. Upsal. III. 1: 107. 1851.[1]

Type: not known to be in existence; not in Herb. Fries, at Upsala, nor in Kew Herb.

Pileus pliant becoming rigid, deeply infundibuliform, 5–7½ cm. broad, radiately rugose, glabrous, fuscous; stem solid, rather glabrous, pallid; hymenium inferior, somewhat rugose, gray.

On the ground. Near San José, Costa Rica.

This species bears so singular a resemblance to *Craterellus cornucopioides* that from pictures they are scarcely to be distinguished. The present species has the stem truly solid and the substance fleshy pliant when living, nearly stony-woody when dry; stem 5–7½ cm. long, 4–6 mm. thick, equal or attenu-

[1] A figure will be given in Part II.

ated at the base, compressed, rather glabrous, very tough, pallid; pileus membranaceous-cartilaginous, when dry quite rigid, radiately rugose, with the ridges elevated towards the undulate and at first fimbriate margin, not zonate after the manner of species of *Stereum;* hymenium inferior, hardened. Related to *Cladoderris.*

I refer to *T. cornucopioides* a collection made in Jamaica by Prof. F. S. Earle, in 1902, the specimens of which agree well with the original description, as translated above, except in size. They are 3–3½ cm. high and 2 cm. broad with stem about 1 cm. long by 2–4 mm. thick. The dried fructification is very hard and stony and softens so little with water that the edge of the razor is turned in sectioning. The spores are colorless and even at first and become slightly colored and angular, 9–10 x 6μ.

Specimens examined:

Jamaica: Castleton Gardens, F. S. Earle, New York Bot. Gard., Plants of Jamaica, 238.

13. **T. vialis** Schw. (Syn. N. Am. Fungi) Trans. Am. Phil. Soc. N. S. 4: 165. 1834. Plate 5. fig. 15.

T. tephroleuca B. & C. Grevillea 1:149. 1873.

Type: in Herb. Schweinitz.

Fructification coriaceous, dirty whitish or pallid, sometimes wood-brown at the center, upper surface usually radiately plicate or rough with masses of agglutinated fibers; pileus polymorphic, sometimes composed of ascending lobes or small pilei which arise from a common base and grow together above to form a broad cup, or sometimes with the whole interior of the cup filled with small pilei and lobes many of which arise proliferously from the upper surface of the outer lobes; stem central when present; hymenium inferior, rugose, somewhat papillose, yellowish pallid becoming avellaneous or somewhat fuscous; spores olive-buff under the microscope, bluntly angular (i. e., tips of the angles obtuse), 4½–7 x 4½–5μ.

Fructification 2½–5 or 6 cm. high, 2½–7 cm. broad.

On ground in frondose woods. Vermont to South Carolina and west to Illinois. September.

This is a fine, large species well marked by the dirty whitish or yellowish, fibrillose upper surface of the pileus, thick substance of the same color unless the specimen is old, and the brown,

slightly wrinkled hymenium. As in the otherwise very different *T. caryophyllea*, large specimens sometimes resemble a double flower from the great number of small pileoli and lobes present in the center. Schweinitz described the species as sometimes having dimidiate pilei, but I have seen no such specimens. My collection assumed a disagreeable odor in drying but no such odor has been noted by others.

Specimens examined:

Exsiccati: Ell. & Ev., N. Am. Fungi, 1110, and Fun. Col., 1593, in both under the name *T. caespitulans*.

Vermont: Lake Dunmore, *E. A. Burt.*

New Jersey: Newfield, *J. B. Ellis* (in Mo. Bot. Gard. Herb., 5155), also in the exsiccati cited.

Pennsylvania: Bethlehem, *Schweinitz*, type (in Herb. Schw.); *Michener, 1504* (in Curtis Herb. and in Kew Herb.), the cotype and type respectively of *T. tephroleuca;* Trexlertown, *W. Herbst, 43, C. G. Lloyd and W. Herbst, 2866, 3088* (both in Lloyd Herb.); *N. M. Glatfelter* (in Mo. Bot. Gard. Herb., 42561).

Dist. of Columbia: Washington, *F. J. Braendle*, comm. by C. H. Peck.

North Carolina: *G. F. Atkinson* (in Cornell Univ. Herb., 23253); Asheville, *H. C. Beardslee;* Schweinitz cited North Carolina as a station.

South Carolina: Caesar's Head, *Ravenel*, one of the types (in Curtis Herb. and Kew Herb.) of *T. tephroleuca*.

Ohio: *C. G. Lloyd, 4000.*

Illinois: Glen Ellen, *E. T. and S. A. Harper, 669.*

14. T. albido-brunnea Schw. Trans. Am. Phil. Soc. N. S. **4**: 166. 1834. Plate 5. fig. 13.

Stereum Micheneri B. & C. Grevillea **1**: 162. 1873 (in part).–*Stereum spongiosum* Massee, Jour. Linn. Soc. Bot. **27**: 172. 1889.–*Thelephora odorifera* Peck, Rep. N. Y. State Mus. **44**: 132 (22). 1891.

Type: in Herb. Schweinitz.

Pileus sessile or with very short stem, coriaceous, spongy when dry, uniformly cinnamon-buff or with the older portions chestnut-brown, sometimes assuming mesopod form when encircling small twigs or shrubs, sometimes effuso-reflexed, usually dimidi-

ate and somewhat imbricated, fibrous-tomentose, margin thick and entire; substance concolorous with the upper surface, spongy, more than 2 mm. thick, with hyphae 4½–5μ in diameter; hymenium inferior, even, not polished, cinnamon-buff; basidia simple; spores deep olive-buff under the microscope, echinulate, 8–10 x 6–8μ.

Pileus 2–4 cm. in diameter when circular, or 1–2½ cm. long, 2–4 cm. broad, often 1 cm. thick at base when dimidiate.

Running up and encircling twigs on the ground and against the base of shrubs. Canada to Louisiana and west to Wisconsin. August.

Peck describes the odor as quite fragrant at first but states that it is lost after a few weeks; I did not notice any especial odor for my collection. *T. albido-brunnea* may be distinguished from our other dimidiate and reflexed species of *Thelephora* by its even and pale hymenium and thick spongy pileus. Schweinitz confused one collection of this species with *T. biennis* Fr., from the specimen of which in the Fries Herbarium, at Upsala, it is clearly distinct. The types of *Stereum spongiosum* Massee, viz., *Curtis, 3582,* and *Ravenel, 1732,* in Kew Herbarium, have colored echinulate spores 8–10 x 6–7μ, although described by Massee as "ellipsoideæ 6–7 x 4μ" without mention of color and projections of the wall. The type of *Thelephora odorifera* Peck, in Coll. N. Y. State, is somewhat bleached or faded but quite typical.

Specimens examined:

Exsiccati: Ravenel, Fun. Car. IV, 12, the type distribution of *T. Micheneri* B. & C.; Ell. & Ev., N. Am. Fungi, 1599, and Fun. Col., 1209, under the name *T. Micheneri* in both.

Canada: Toronto, *J. Dearness* (in Lloyd Herb.).

Vermont: Lake Dunmore, *E. A. Burt.*

New York: Selkirk, *C. H. Peck* (in Coll. N. Y. State), the type of *T. odorifera* Pk.; Alcove, *C. L. Shear, 1010, 1163, 1184;* Jamesville, *L. M. Underwood.*

Pennsylvania: Bethlehem, *Schweinitz* (in Herb. Schw.), the type, and also the Nos. 627 and 625 of Syn. N. Am. Fungi under the names respectively of *T. biennis* and *T. laciniata; Michener* (in Curtis Herb., 3582, and also in Kew Herb., same number), type of *Stereum spongiosum* Massee; Trexlertown, *W. Herbst, 18,* and (in Lloyd Herb., 3052).

North Carolina: Blowing Rock, *G. F. Atkinson, 4322.*
South Carolina: *Ravenel, 790* (in Curtis Herb. and in Kew Herb.), under the name *Thelephora biennis;* Santee Canal, *Ravenel, 1732* (in Curtis Herb. and in Kew Herb.), type of *Stereum spongiosum* Massee.
Louisiana: Bogalusa, *C. J. Humphrey, 466.*
Ohio: Cincinnati, *A. P. Morgan,* Lloyd Herb., 2627.
Michigan: Saugatuck, *E. A. and S. A. Harper, 654.*
Wisconsin: Milwaukee Co., comm. by Mrs. F. W. Patterson.

15. T. lutosa Schw. Trans. Am. Phil. Soc. N.S. 4: 166. 1834.[1]
Type: in Herb. Schweinitz.

Pilei cespitose, densely imbricated, at first somewhat fleshy but at length hard, undulate-plicate, yellowish, almost subtomentose with pulverulence, somewhat horizontally attenuated behind, margin sublobate, at length inflexed; pileus less than 2 mm. thick, with hyphae 3μ in diameter; hymenium becoming yellowish, even; spores olive-buff under the microscope, angular, 5–6 x 3½–4μ.

Cluster about 1½ cm. high and broad.

On the ground in roads and in woods. North Carolina.

The type is distinct from *T. albido-brunnea,* having thinner pileus, finer hyphae, and smaller and paler spores. The pilei were crowded together into a small buff-colored cluster about 1½ cm. high and broad, somewhat as in *Tremellodendron pallidum* (Schw.); I failed to find stems at their bases.

Specimens examined:
North Carolina: Salem, *Schweinitz* (in Herb. Schw.), type.

16. T. cuticularis Berk. Hooker's Lond. Jour. Bot. 6: 324. 1847. Republished in Lea, Catalogue of Plants in Vicinity of Cincinnati 66. *d.* 1849. Plate 5. fig. 14.

Type: in Kew Herb., and a portion of it from Berkeley in Curtis Herb.

Pilei coriaceous-soft, effuso-reflexed or dimidiate, imbricate, sometimes laterally confluent, drying pinkish buff to cinnamon-brown, with a broad, pale margin, surface radiately rugose, soft, silky fibrillose; substance of the same color as pileus; hymenium inferior, concave, even, drab to brownish drab; spores umbrinous under the microscope, flattened on one side or somewhat kidney-shaped, not angular, echinulate, 8–9 x 6–7μ.

[1] A figure will be given in Part II.

Pileus 1–1½ cm. long, 2–4 cm. broad, 1 mm. thick.

On mossy bark at the base of trees and on fallen twigs in groves. Vermont to Texas and west to Missouri. June to August.

In his description Berkeley noted that the odor of this species is strong and unpleasant; my specimens retained such an odor for several years but I did not notice it before they were dried. *T. cuticularis* may be distinguished from our other species by its drab hymenium, portions of which become sage-green when crushed under a cover glass in a 7 per cent solution of potassium hydrate, and by its spores, which are not at all angular or irregular as regards the main body of the spore, but ovoid and flattened on one side or slightly kidney-shaped and sparingly studded with slender spines.

Specimens examined:

Vermont: Middlebury, *E. A. Burt.*

Rhode Island: *Olney, 1851* (in Kew Herb. and in Curtis Herb.).

Pennsylvania: Bethlehem, *Schweinitz* (in Herb. Schw.), the Nos. 628 and 629 of Syn. N. Am. Fungi, under the names respectively of *T. fuscocinerea*, and *T. gausapata;* Kitanning, *D. R. Sumstine, 1.*

Delaware: Newark, *H. S. Jackson.*

North Carolina: Asheville, *H. C. Beardslee, 03195.*

Florida: *Mrs. Sams*, comm. by C. G. Lloyd.

Texas: *W. H. Long, Jr., 351, 387* (in Cornell Univ. Herb.).

Ohio: Waynesville, *T. G. Lea* (in Kew Herb.), type; Preston, *A.P. and L. V. Morgan*, comm. by C. G. Lloyd, also *C. G. Lloyd*, specimen dated July 26, 1896; Cincinnati, *C. G. Lloyd, 4492.*

Wisconsin: Blue Mounds, *E. T. and S. A. Harper, 861.*

Missouri: Columbia, *B. M. Duggar, 289.*

17. **T. intybacea** Pers. ex Fries, Syst. Myc. 1: 431. 1821.
Plate 5. fig. 11.

T. intybacea Pers. Syn. Fung. 567. 1801–1807; Myc. Eur. 1: 110. 1822.

Illustrations: Bulliard, Champ. de la France *pl. 278.*–Bigeard et Guillemin, Champ. Super. France 436. *pl. 44. f. 1.*

Fructifications cespitose, soft, whitish, then rufous-ferruginous, drying chestnut-brown to Rood's brown, with stems

somewhat lateral and growing into one another; pilei imbricated, fibrous, usually with the fibers matted and agglutinated into appressed and wholly adnate squamules, margin dilated and whitish-fimbriate at first, at length becoming entire and colored like the rest of the pileus; hymenium inferior, concolorous with the upper surface, papillose; spores concolorous with hymenium, snuff-brown under the microscope, angular-tuberculate, 7–9 x 6–8μ.

Clusters often 5–8 cm. in diameter; individual pileus 2–3 cm. long, 2–4 cm. broad, 1 mm. thick.

On the ground in pine woods, growing up from the layer of fallen leaves. Ontario to North Carolina and westward to Ohio and Michigan. August to October.

The clusters are sometimes central but more often with the pilei lateral and triangular; sometimes the mass ascends small sticks and then extends out from this support in reflexed forms; the upper surface is usually uneven and dries somewhat depressed between the adnate squamules. This species is distinguished from ferruginous specimens of *T. terrestris* by the thicker and entire margin of the pileus and by the absence of free squamules.

Specimens examined:

Exsiccati: Ell. & Ev., Fun. Col., 1410.

Austria: *G. Bresadola.*

Ontario: Toronto, *J. Dearness,* comm. by C. G. Lloyd; Harraby, Lake Rosseau, *E. T. and S. A. Harper, 682.*

Maine: Portage, *L. W. Riddle, 3.*

New Hampshire: Shelburne, *W. G. Farlow.*

Vermont: Middlebury, Sudbury, Grand View Mt., *E. A. Burt.*

Massachusetts: *A. P. D. Piguet,* comm. by Dr. Farlow; Natick, *G. E. Morris, No. E.*

Connecticut: East Hartford, *C. C. Hanmer, 1434.*

New York: Alcove, *C. L. Shear, 1009;* East Galway, *E. A. Burt;* Ithaca, *G. F. Atkinson,* Cornell Univ. Herb., 3050, 19652.

Dist. of Columbia: Takoma Park, *C. L. Shear, 799, 796;* Washington, *O. F. Cook, 4,* comm. by P. L. Ricker.

North Carolina: Asheville, *H. C. Beardslee, 0341.*

Ohio: *A. P. Morgan* (in Lloyd Herb.).

Michigan: *C. G. Lloyd, 4546;* Lawton, *L. A. Hawkins;* Sailor's Encampment, *Allen and Stuntz, 1,* Univ. of Wis. Herb.

18. **T. terrestris** Ehrh. ex Fries, Syst. Myc. 1: 431. 1822. Plate 5. fig. 10.

T. terrestris Ehrh. Crypt. Exsicc. No. 178. 1785.–Persoon, Syn. Fung. 566. 1801; Myc. Eur. 1: 113. 1822.–*Stereum laciniatum* Pers. Obs. Myc. 1: 36. 1796.–*Thelephora laciniata* Pers. Syn. Fung. 567. 1801.–*T. caryophyllea β laciniata* Pers. Myc. Eur. 1: 112. 1822.–*T. laciniata* Fries, Syst. Myc. 1: 431. 1821.

Illustrations: Batsch, Elenchus Fung. *pl. 24. f. 121.*–Nees, System der Pilze *pl. 34. f. 251.*–Bolton, Hist. Fung. *pl. 173.*–Sowerby, Col. Fig. of Eng. Fungi *pl. 213.*–Cooke, Handbook 1: 310.–Stevenson, Brit. Hym. 2: 261.–Smith, Brit. Basid. 399. *f. 96 C-E.*

Fructifications dark fuscous to fawn-color, coriaceous-soft, cespitose, obconic, with a short stem-like base, or dimidiate and sessile, or incrusting and effuso-reflexed; pileoli more or less imbricated, sometimes laterally confluent, fibrous-squamulose and usually strigose, thin, margin fibrous-fimbriate and laciniate; hymenium inferior, papillose, fuscous to fawn-color; spores pale fuscous, irregular, angular, sometimes slightly tuberculate, 6–9 x 6μ.

Clusters 5–8 cm. in diameter, with single pileolus about 3 cm. long and broad; obconic pileus 2–3 cm. in diameter; dimidiate pileolus 1½–2 cm. long, 2–3 cm. broad, about 1 mm. thick.

On sandy ground in bare fields and at base of trunks and from fallen twigs and leaves in pine woods. Canada to South Carolina, and in Michigan, Jamaica, and Alaska. July to December.

My observations of this species acquired from specimens received and from seeing it growing abundantly near Middle Grove, N. Y., seem to show that the medium from which this fungus derives its food produces an interesting effect on the fructification. Growing from bare, sandy ground the fructifications are dark fuscous in color, and may be flattened clusters of imbricated pileoli, or of the obconic-pileus type composed of ascending pileoli confluent laterally, or dimidiate, sessile pileoli. When growing on abundant woody matter, as is the case in the specimen in Sowerby's illustration already cited, the fructification assumes a redder color and replaces its dimidiate, sessile pileus on earth by a reflexed one on the wood. With regard to

other forms of the clusters and pileoli, the covering of the upper surface, and the spore characters there is no difference between those fructifications produced without woody food and those having it. There is no sharp color separation between these color extremes.

Specimens growing on the ground usually have a short stem-like base, while those growing on wood are reflexed; the same collection may show both these conditions, as, for example, that from Skagway, Alaska, if some of the fructifications start from sticks and others directly from the ground. Persoon regarded the stem in *T. terrestris* as the chief character separating that species from his *T. laciniata*, as may be seen from his own descriptions contrasting the two in his 'Synopsis Fungorum,' pp. 566 and 567, as follows:

"3. THEL. TERRESTRIS: subimbricata obscure fusca, pileo applanato fibroso-strigoso."

"Hab. in arenosis ad terram. *Stipes* breuis, lateralis omnino adest. Substantia submollis, non ita coriacea sicca, vti in ceteris speciebus."

"4. THEL. LACINIATA: imbricata obscure fusca, pileo tenui laciniato crispo subtus papillis congestis scabro."

"Hab. ad radices truncorum. Cespitem difformem efformat, 2 vnc. lata, tenuis. *Stip.* vix adest distinctus."

These descriptions supplement each other as a description for one species; each has special application to fructifications growing side by side under such conditions as to show that they are from a common mycelium. Persoon never claimed that his species differed from *T. terrestris* in color. Fries gave a different description of *T. laciniata* in his works cited—to the injury of *T. intybacea*—, but the characters he gives are not satisfactory. European mycologists with a wide knowledge of the *Thelephoraceæ* as they grow are unable to distinguish these two species. In letters to me, Bresadola regards *T. laciniata* as a synonym of *T. terrestris;* and Romell does not know *T. terrestris* if it is distinct from *T. laciniata.*

Specimens examined:

Exsiccati: Ellis, N. Am. Fungi, 511; Ell. & Ev., N. Am. Fungi, 2732, under the name *T. intybacea.*

Austria: *G. Bresadola.*

Sweden: *G. Romell, 52, 55, 56, 57.*

Newfoundland: *A. C. Waghorne, 276* (in Mo. Bot. Gard. Herb.).

Quebec: Gaspe, *J. Macoun, 229.*

Ontario: Ottawa and Belleville, *J. Macoun.*

Maine: Wells, *J. Blake,* comm. by P. L. Ricker.

New Hampshire: Chocorua, *W. G. Farlow.*

Massachusetts: Magnolia and Woods Hole, *W. G. Farlow;* Ipswich, *G. E. Morris, No. F.*

Connecticut: South Windsor, East Hartford, and Rockville, *C. C. Hanmer, 1227–29, 944, 1057.*

New York: East Galway and Middle Grove, *E. A. Burt,* three collections from the latter station; Ithaca, *G. F. Atkinson,* Cornell Univ. Herb., 22976.

New Jersey: Belleplain, *C. L. Shear, 1246;* Newfield, *J. B. Ellis,* Ellis, N. Am. Fungi, 511.

Pennsylvania: *Schweinitz* (in Herb. Schw.), the 624 of Syn. N. Am. Fungi.

North Carolina: Asheville, *H. C. Beardslee, 02280;* Salem, *Schweinitz* (in Herb. Schw.), the 624 of Syn. N. Am. Fungi.

Alabama: Tuskegee, *Beaumont, 199* (in Curtis Herb.).

South Carolina: Society Hill, *M. A. Curtis, 2693* (in Curtis Herb.).

Michigan: Agricultural College, *G. H. Hicks,* Ell. & Ev., Fun. Col., 2732.

Alaska: Skagway, *J. Macoun, 47; Evans, 410* (in Mo. Bot. Gard. Herb.).

Jamaica: Cinchona, *W. A. and E. L. Murrill,* New York Bot. Gard., Fun. of Jamaica, 451.

19. **T. griseozonata** Cooke, Grevillea **19**: 104. 1891.
Plate 5. fig. 12.

Type: in Ravenel, Fun. Amer., 444.

Fructifications cespitose, coriaceous-soft; pileoli extended into a short sublateral stem, imbricate, applanate, silky-strigose, zonate with alternating cervine (Rood's brown) and light buff zones, margin subfimbriate; hymenium inferior, castaneous when fresh, drying Rood's brown, rugose, somewhat papillose; spores pale fuscous, angular, 6–9 x 6–7μ.

Cluster 3–6 cm. in diameter; obconic pileus and single pileolus each 2–3 cm. in diameter.

On sandy ground in pine woods. New Jersey to Louisiana. August to November.

This species is closely related to *T. terrestris* and has the same habitat, habit of growth, and spore characters, but is distinguished from that species by its zonate pileus. The fructifications usually occur in flattened clusters with spreading pileoli; sometimes the individual pileoli acquire an infundibuliform appearance by the growing together for part of their length of opposite edges of individual pileoli; sometimes a small obconic pileus occurs composed of two or more pileoli with adjacent edges confluent. In the collection cited below from Mississippi, small lobes are present in the cavity of the cup, as in *T. vialis* and *T. caryophyllea*.

Specimens examined:

Exsiccati: Ravenel, Fungi Am., 444, type distribution; Ravenel, Fun. Car. II, 28, under the name *T. caryophyllea;* Ellis, N. Am. Fungi, 714; Ell. & Ev., Fun. Col., 1305.

New Jersey: Newfield, *J. B. Ellis*, in his exsiccati cited.

South Carolina: Aiken, *H. W. Ravenel*, Fungi Am., 444, type collection.

Alabama: Auburn, *C. F. Baker*, Lloyd Herb., 3462.

Mississippi: Biloxi, *Mrs. E. S. Earle, 32.*

Louisiana: St. Martinville, *A. B. Langlois, by.*

20. T. fimbriata Schw. ex Schweinitz, Trans. Am. Phil. Soc. N. S. **4**: 166. 1834. Plate 4. fig. 3.

Merisma fimbriatum Schw. (Syn. Fung. Car., No. 1067) Schrift. d. Naturforsch. Gesell., Leipzig, **1**: 110. 1822.–*Thelephora scoparia* Peck, Rep. N. Y. State Mus. **42**: 123 (27). *pl. 2. f. 20, 21.* 1889.

Illustrations: Peck, Rep. N. Y. State Mus. **42**: *pl. 2. f. 20, 21.*

Type: in Herb. Schweinitz.

Fructification coriaceous-soft, incrusting and ascending small plants (mosses, etc.), here and there emitting fascicles of branches united below, subterete, acuminate or fimbriately incised, at first pale or whitish, soon ferruginous brown, drying Rood's brown; hymenium even, pruinose-pubescent; spores umbrinous, tuberculate, 7–11 x 6–9 μ.

Incrusting and ascending upward 1–3 cm.; free branches 5–10 mm. long, 1 mm. thick, sweep of fascicle about 5–10 mm.

In moist places. New York to South Carolina, and west to Illinois. July and August.

The type is an incrusting specimen, covering as its main axis a small twig in one specimen and a moss in the other, and sending out a few lateral branches which are flattened towards the free ends and subfimbriate; main trunk is cylindric, latericius (of 'Chromotaxia'), ends of branches paler; spores umbrinous under the microscope, tuberculate, 7–8 x 6 μ. Schweinitz described the species as becoming hard and cartilaginous, but this is an error probably due to the foreign matter surrounded by the main trunk. Several other specimens are present in his herbarium under various names.

Specimens examined:

Exsiccati: Ellis, N. Am. Fungi, 512, under the name *T. cristata.*

Massachusetts: Weston, *A. B. Seymour, T 1* (in Mo. Bot. Gard. Herb., 45573).

New York: Bethlehem and Selkirk, *C. H. Peck* (in Coll. N. Y. State), type of *T. scoparia;* Syracuse, from Herb. Cornell Univ., 19474.

New Jersey: Newfield, *J. B. Ellis,* N. Am. Fungi, 512.

Pennsylvania: Bethlehem, *Schweinitz* (in Herb. Schw.), the 615 of Syn. N. Am. Fungi, under the name *T. stabularis.*

North Carolina: Salem, *Schweinitz* (in Herb. Schw.), type, and also the 1063 of Syn. Fung. Car., under the name *Merisma fuscescens.*

Indiana: Millers, *E. T. and S. A. Harper, 670.*

Illinois: Havana, *H. C. Beardslee;* Riverside, *E. T. and S. A. Harper, 668.*

21. T. perplexa Burt, n. sp.[1]

Type: in Curtis Herb.

Fructification incrusting, coriaceous, consisting of a resupinate membrane from the central portion of which arise cylindric trunks either simple or digitately branched; resupinate portion spongy, firm, separable, fuscous at the center, margin thin, determinate, pinkish buff; ascending portions spongy, firm,

[1] A figure will be given in Part II.

fuscous, simple and tapering upward or soon branching and terminating in paler either subulate tips or somewhat flattened ends; spores fuscous, subglobose, echinulate, 8–10 x 8–9μ.

The resupinate membrane may be 3 cm. in diameter; ascending portion of fructification 2–3 cm. high, 1½–2 mm. thick.

On decaying leaves and sticks on the ground. Cuba.

Berkeley & Curtis based their description of *Thelephora dentosa* on two collections made in Cuba by C. Wright in different years; these collections are different specifically. The original description applies chiefly to the earlier collection, made in 1857, which is unnumbered. I take my type of *T. perplexa* from the later collection, *C. Wright, 238*.

Specimens examined:

Exsiccati: Fungi Cubenses Wrightiani, *C. Wright, 238*, under the name *Thelephora dentosa* B. & C.

Cuba: *C. Wright, 238*, type (in Curtis Herb.).

22. T. dentosa Berk. & Curtis emend Burt.[1]

T. dentosa B. & C. (Fungi Cubenses) Jour. Linn. Soc. Bot. 10: 329. 1867.

Type: type and cotype in Kew Herb. and Curtis Herb. respectively.

Fructification coriaceous-soft, incrusting leaves and small twigs on the ground and ascending as free, sessile, dilated, triangular, flabelliform pilei which are dentate at the upper end or deeply divided into a few finger-shaped divisions, honey-yellow to tawny olivaceous throughout, minutely hairy under a lens; spores honey-yellow, globose to ovoid, weakly echinulate, 6–10 x 6–8μ.

Pileus 1 cm. high, 5 mm.–1 cm. broad.

On rotten vegetation. Cuba. June.

As already stated in connection with *T. perplexa*, Berkeley & Curtis cited for types of their *T. dentosa* specimens from two collections made in Cuba by C. Wright. These collections were made with an interval of several years between the collections, which differ specifically. As noted by Berkeley & Curtis, their description applies better to the earlier collection, to which I now

[1] A figure will be given in Part II.

restrict their species. This earlier collection was distributed by C. Wright, unnumbered, under the name *Thelephora dentosa* B. & C. before the publication of the description of this species, and the cotype in Curtis Herb. is unnumbered also. By what was apparently a slip of the pen, Berkeley cited this type as *C. Wright, 507.* By the kindness of Dr. Farlow I have been permitted to examine the manuscript records which show that Wright collected only one No. 507, which was determined by Berkeley as *Xylaria obovata* Berk. and is cited under this species by Berk. & Curtis, Jour. Linn. Soc. Bot. **10**: 380. 1867. I find in Curtis Herb. such a specimen labelled *Xylaria obovata* Berk., Cuba, *C. Wright, 507.* I conclude that the type and cotype of *T. dentosa* B. & C., first cited in their description, are from the collection distributed by C. Wright, unnumbered, under the name *Thelephora dentosa* B. & C.

Specimens examined:

Exsiccati: Plantae Cubenses Wrightianae, unnumbered, under the name *Thelephora dentosa* B. & C.

Cuba: *C. Wright,* cotype (in Curtis Herb.).

23. T. spiculosa Fries, Syst. Myc. **1**: 434. 1821; Epicr. Syst. Myc. 539. 1836–38. Plate 4. fig. 2.

Illustrations: Persoon, Syn. Fung. *pl. 3. f. 16.*

Type: an authentic specimen from Fries, in Kew Herb.

Fructifications cespitose, from byssoid becoming fleshy, variable by incrusting habit, pale buff at first, main portions becoming purplish-fuscous (Rood's brown) with age, ramose-spiculous, tips penicillate and whitish; spores umbrinous under the microscope, irregular, echinulate, 8–9 x 6–7 μ.

Clusters 1–2 cm. high, 2–4 cm. in diameter, single fructification 1–2 cm. high, about 1 mm. in diameter, with branches spreading 4–6 mm.

On leaves on ground in moist groves. Ohio to Wisconsin. July. Rare.

The best specimens which I have seen have main trunks of the fructifications running side by side over partially decayed beech leaves and confluent into an effused mass. These trunks ascend obliquely from the leaves to a height of 1–2 cm., branch sparingly, and terminate in spiculous tips. The fructification

must be inconspicuous in the woods since the general color of the mass is the same as that of the leaves on which it is effused, although the main trunks may be darker.

Specimens examined:

Exsiccati: Kunze, Fun. Sel. Exsic., 560.
Sweden: specimen from Fries (in Kew Herb.).
Austria: *G. Bresadola.*
Ohio: Preston, *C. G. Lloyd.*
Michigan: Glen Lake, *C. G. Lloyd, 02471.*
Wisconsin: Lake Geneva, *E. T. and S. A. Harper, 883.*

(To be continued.)

Explanation of Plate

PLATE 4

All figures of plates 4 and 5 have been reproduced natural size from photographs of dried herbarium specimens of species of *Thelephora.*

Fig. 1. *Thelephora anthocephala.* From specimen collected at Linwood, Ohio, by C. G. Lloyd, No. 02164.

Fig. 2. *T. spiculosa.* *a*, from specimen on leaves of *Fagus* collected in Europe by Bresadola, which I compared with the specimen from Fries in Kew Herbarium; *b*, from specimen collected at Glen Lake, Mich., by C. G. Lloyd, No. 02471.

Fig. 3. *T. fimbriata.* From specimen incrusting living strawberry (*Fragaria*) plant, collected at Riverside, Ill., by E. T. and S. A. Harper, No. 668.

Fig. 4. *T. palmata.* From specimen from New Jersey, from C. G. Lloyd, No. 4612.

Fig. 5. *T. magnispora.* From type specimens collected at Chester Vale, Jamaica, by W. A. and Edna L. Murrill, No. 295. *a* shows upper surface and side of pileus, and *b*, the hymenium.

Fig. 6. *T. regularis.* From a sketch of the type in Herb. Schweinitz.

Fig. 7 *a*. *T. multipartita.* From specimens collected at Trexlertown, Pa., by Dr. W. Herbst.

Fig. 7 *b*. *T. regularis.* From specimens collected at Clayton, Del., by H. S. Jackson.

Fig. 8. *T. scissilis.* From type specimens collected at Bingen, Wash., by W. N. Suksdorf, No. 716.

Fig. 9. *T. caryophyllea.* From specimens collected in Michigan, by C. G. Lloyd, No. 4547.

BURT—THELEPHORACEAE OF NORTH AMERICA

1. THELEPHORA ANTHOCEPHALA.—2. T. SPICULOSA.—3. T. FIMBRIATA.—4. T. PALMATA.—5. T. MAGNISPORA.—6 AND 7 b. T. REGULARIS.—7 a. T. MULTIPARTITA.—8. T. SCISSILIS.—9. T. CARYOPHYLLEA.

COCKAYNE, BOSTON.

Explanation of Plate

PLATE 5.

Fig. 10. *T. terrestris.* From specimens collected on ground in open fields at Middle Grove, N. Y. *a* shows the fibrose-strigose upper surface and fimbriate margin of the pileus, and *b*, the hymenium of lower surface.

Fig. 11. *T. intybacea.* From specimens collected in pine woods incrusting fallen pine leaves and twigs at Middlebury, Vt. *a* shows upper surface with matted, adnate squamules and whitish, thick, entire margin; *b*, the hymenium of lower surface.

Fig. 12. *T. griseozonata.* From specimen of type collection, distributed in Ravenel, Fun. Amer., No. 444.

Fig. 13. *T. albido-brunnea.* *a*, upper side of specimen collected at Saugatuck, Mich., by E. T. and S. A. Harper, No. 654. The specimen is about 2 cm. thick; *b*, hymenium of specimen collected at Lake Dunmore, Vt.

Fig. 14. *T. cuticularis.* From specimens collected at Blue Mounds, Wis., by E. T. and S. A. Harper, No. 861. *a*, viewed obliquely from above; *b*, viewed from under side to show hymenium.

Fig. 15. *T. vialis.* From specimen collected at Lake Dunmore, Vt.

BURT — THELEPHORACEAE OF NORTH AMERICA

10. THELEPHORA TERRESTRIS. — 11. T. INTYBACEA. — 12. T. GRISEO-ZONATA. — 13. T. ALBIDO-BRUNNEA. — 14. T. CUTICULARIS. — 15. T. VIALIS.

COCKAYNE, BOSTON.

INDICATIONS REGARDING THE SOURCE OF COMBINED NITROGEN FOR ULVA LACTUCA

G. L. FOSTER

Teaching Fellow in the Henry Shaw School of Botany of Washington University

Introduction

Very little attention has been given the question of the sources of nitrogen for marine algæ. Nevertheless, the question is an interesting one both physiologically and ecologically, because of the extremely small amount of nitrogen supposed to be present in sea-water, and because of the very noticeable change in the type of algal flora when the nitrogen content of the environment is increased, as by the presence of sewage. The literature bearing on the subject is practically limited to a debate between a few authors as to the amount and form of nitrogen in sea–water, and the way in which the supply is maintained. This dispute involves some questions of fundamental importance for marine biology; consequently, a brief statement of the different views is pertinent.

Natterer (13) reports that careful analyses of water from the high seas show scarcely a trace of nitrates. Nitrites are somewhat more abundant, but not sufficiently so to admit of quantitative determination. Ammonium compounds, on the other hand, according to Thoulet, are present in sufficient amount to be quantitatively determined, and vary from .13 to .34 mg. per liter (.013–.034 per cent) according to the locality. Reinke (15) considers these amounts of nitrogen reported to be insufficient for the production of the enormous amount of living material in the sea, especially when the activity of nitrifying and denitrifying bacteria is taken into account. He considers as of prime importance in this question the nitrogen-fixing bacteria which have been demonstrated in sea-water by Benecke and Keutner (4), and others. Reinke found *Azotobacter* embedded in the gelatinous material on the surface of *Laminaria* fronds and argues for a symbiotic relation between the algæ and bacteria.

Brandt (7), however, attaches little or no importance to Reinke's view, and maintains that the nitrogen content of sea-water is determined by a balance between the activity of denitrifying bacteria, on the one hand, and the great amount of nitrogenous material carried to the sea by the rivers, on the other. Brandt (5, 6) considers that the nitrogen content of sea-water is at a "minimum" and is the limiting factor in the production of marine organisms. Considering especially the plankton life, he finds that the amount of plankton is proportional to the nitrogen content of the water, and correlates the comparative poverty of tropical seas in plankton life with the relatively greater activity of denitrifying bacteria in the warmer waters.

More recently Pütter (14) has reported that analyses of the water from the Gulf of Naples give per liter .18 mg. of nitrogen in nitrates and nitrites, and .56 mg. in ammoniacal nitrogen. Furthermore, he claims that these figures represent less than half the total combined nitrogen actually present in sea-water. In his opinion there is no need for considering the nitrogen content to be at a "minimum" since it is present in greater concentration than the carbon dioxide. It is impossible to say which of these views is the correct one, and further work in this field is much needed.

The above named authors incidentally assume that nitrogen is available for the algæ only in the form of nitrates or ammonium salts. This is entirely an a priori assumption, as no data are offered in support of such a view. On the contrary, it seems more likely that the algæ can use many organic nitrogen compounds. This would seem probable in the light of recent work which has been done on the fresh-water algæ.

In regard to the nutrition of the fresh-water forms we have departed far from the old idea that green plants are strictly autotrophic. Thus, by the work of Beyerinck (3), Charpentier (8), Chick (9), Artari (1, 2), and others, it has been established that many of the fresh-water algæ have, with respect to nitrogen, distinct saprophytic tendencies,—preferring organic to inorganic nitrogen. Artari, especially, has shown that several algæ (*Chlamydomonas*, *Stichococcus*, *Chlorella*, *Scenedesmus*, and others) can grow and retain the chlorophyll under completely

saprophytic conditions, as in solutions containing amino acids and glucose in the absence of light and carbon dioxide. In all these cases, however, growth is more rapid under so-called mixotrophic conditions, i. e., with both organic nitrogen and carbon present in addition to sunlight and carbon dioxide. Artari takes up the question of the relative value of different nitrogenous compounds, and shows that they vary greatly with different algæ,—some preferring peptones, others, amino acids and ammonium salts, and a few, nitrates. On the whole, the majority of forms investigated grow best in the presence of amino nitrogen. Many algæ, especially of this last class, are often found in water polluted with sewage or decaying organic matter.

Among the marine algæ, there is a more or less definite flora characteristic of sewage-polluted waters. Most conspicuous among the plants of this group are the species of *Ulva*. Letts and Richards (11), in their reports on sewage in British harbors, state that *Ulva latissima* grows in excessive quantities in polluted waters, and they find that the nitrogen content of this seaweed varies with the degree of pollution of the water. Cultural experiments conducted by Letts and Richards showed that *Ulva latissima* grows more rapidly in a mixture of sewage and sea-water than in pure sea-water alone.

Experimental

Preliminary experiments were made at the Woods Hole Laboratory to determine the sources of available nitrogen for *Ulva lactuca*. The algal material used in the experiments was collected at the mouth of an inlet where the water was at all times highly polluted with sewage. The cultures were maintained in the laboratory in glass tumblers containing 150 cc. of solution. When brought in, the fronds of *Ulva* were well rinsed in clean sea-water and cut into strips exactly 3 cm. in length and about 2 cm. wide. Three such strips were placed in each vessel, and the cultures kept at a temperature of 21°C. by placing the vessels in a tray of running water. In each case the solution was renewed at the end of 5 days. After 10 days the strips were again measured and the increase in length recorded.

Two main types of nutrient solution were used,—one (solution A) being natural sea-water, the other (solution B) being an artificial sea-water minus nitrogen. These stock sea-waters were made double strength and subsequently diluted by the addition of distilled water and the stock solution of the nitrogenous compounds to be tested. The following nitrogen compounds were used in the experiments: ammonium nitrate, urea, acetamid, sodium asparaginate, acetanilid, and dimethylanilin. Parallel experiments were run, adding these compounds to solution A and solution B.

Preliminary tests roughly determined the maximum non-toxic concentrations of these compounds when added to sea-water to be:

Ammonium nitrate	0.011 gram molecular
Urea	0.010 gram molecular
Acetamid	0.250 gram molecular
Asparagin	0.080 gram molecular

The table presents the results of the experiments. All figures for concentrations represent fractions of gram molecules per liter, except in the case of dimethylanilin. Here the solubility was not known and the figures represent fractions of a saturated solution in distilled water at 20°C. In the column headed "growth" is recorded the increase in length in millimeters of the strips of *Ulva* after 10 days in the solution. In each case the figures for growth represent the average of three or more cultures. Checks show the growth in solutions A and B with no additional nitrogen.

It is apparent from the following table that, under the conditions of the experiment, ammonium nitrate and urea are considerably better nutrients for *Ulva* than the other compounds used. These two cause a marked increase in growth over that of the controls, in both the artificial and natural sea-waters. The nutritive value of these compounds was also indicated by the healthy appearance of the cultures. The algæ were of a deep green color, very turgid, and considerably curled by rapid growth. Judging from the growth and general appearance of the cultures, there is little choice between the nutrient values of ammonium nitrate and urea.

COMPARATIVE TABLE SHOWING GROWTH OF STRIPS OF ULVA IN VARIOUS NITROGEN-CONTAINING SOLUTIONS

Ammonium nitrate			Urea			Acetamid		
Conc.	Growth		Conc.	Growth		Conc.	Growth	
	Sol.* A	Sol.* B		Sol.* A	Sol.* B		Sol.* A	Sol.* B
Check	0.8	0.3	Check	0.8	0.3	Check	0.8	0.3
0.00005	1.0	0.5	0.0005	1.4	0.5	0.001	0.8	0.4
0.0001	1.4	1.5	0.001	1.6	0.6	0.005	1.0	0.4
0.0005	1.9	2.0	0.005	1.6	1.4	0.01	0.7	0.5
0.001	1.4	1.2	0.01	0.9	1.3	0.10	1.0	1.0

Sodium asparaginate			Acetanilid			Dimethylanilin		
Conc.	Growth		Conc.	Growth		Conc.†	Growth	
	Sol.* A	Sol.* B		Sol.* A	Sol.* B		Sol.* A	Sol.* B
Check	0.8	0.3	Check	0.8	0.3	Check	0.8	0.3
0.002	0.7	0.6	0.0005	0.6	0.5	0.002	0.3	0.4
0.01	0.9	0.2	0.0025	0.0	0.0	0.02	0.0	0.5
0.05	0.7	0.0	0.0125	0.0	0.0	0.10	0.0	0.0

*Sol. A = natural sea-water; sol. B = artificial sea-water.

† Conc. under dimethylanilin represents fractions of a saturated solution in distilled water at 20°C.

Acetamid has a somewhat lower nutrient value than ammonium nitrate or urea, but still it causes a greater growth than do the control solutions to which no foreign nitrogen was added. The alga in acetamid solutions appeared normal in every way. The results with the sodium asparaginate were rather unexpected. This compound is well known to be a good nutrient for many fungi and fresh-water algæ. For *Ulva*, on the other hand, sodium asparaginate appears to have no appreciable nutrient value. In no case did it cause any notable increase in growth, although the algal material appeared perfectly normal.

Acetanilid and dimethylanilin are in a separate class,—being decidedly toxic at all the concentrations used. At the lowest concentrations there was slight growth at first, but in ten days all cultures were dead and discolored. The results with these last two compounds are comparable to those obtained with similar substances by Czapek (10) and by Lutz (12) working on fungi and fresh-water algæ. They found that compounds having the nitrogen attached directly to a benzene nucleus are toxic.

Pure culture methods were not attempted on account of the brief time available for this work, and the question of the possible interaction of ammonifying bacteria is therefore pertinent. However, the rapid augmentation of growth upon the addition of the amido compounds, and the comparative absence of bacteria both suggest a direct absorption of these substances. Moreover, since rapid growth of the alga occurs in concentrations of the amido compounds considerably greater than the toxic limit for ammonium salts, and since, further, no evidence of toxicity of fairly strong solutions of urea and acetamid developed during the interval of these experiments, no support is given to the thought that ammonification may be an important factor. However, in further continuation of this work it is proposed to control this possibility by quantitative tests.

It seems probable from the facts brought out here, as well as from the work of Letts and Richards, that *Ulva* is not limited to an inorganic nitrogen supply, since growth occurs with urea or acetamid as the sole source of nitrogen, and, as Letts and Richards have shown, that it grows more rapidly in sewage-polluted water than in pure sea-water. Undoubtedly, further experiments would show that other organic compounds can supply available nitrogen for *Ulva*.

The results also indicate that for *Ulva*, at least, the amount of available nitrogen in the water is the limiting factor in growth. This is shown by the fact that growth is more rapid in sea-water containing additional nitrogen (ammonium nitrate, or urea) than in pure sea-water. The above mentioned results of Letts and Richards also point to the same conclusion, as does

the abundant growth of *Ulva* in nature in waters polluted with sewage.

In conclusion, the writer is pleased to express his thanks for the generous assistance given during this study by Prof. B. M. Duggar, under whose direction the work was carried out while occupying a research table maintained by Dartmouth College at the Marine Biological Laboratory, Woods Hole, Massachusetts.

Graduate Laboratory, Missouri Botanical Garden.

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THE EFFECT OF CERTAIN CONDITIONS UPON THE ACIDITY OF TOMATO FRUITS

B. M. DUGGAR

Physiologist to the Missouri Botanical Garden
Professor of Plant Physiology in the Henry Shaw School of Botany of Washington University

AND M. C. MERRILL

Research Assistant to the Missouri Botanical Garden

In a recent communication the senior author (4) has referred to the possibility that the total acid content of tomato fruits ripened at a temperature of 30°C., or above, may be related in some way to the failure of lycopersicin development at that temperature. It was determined that the "total acidity for green, ripening, and ripe fruits, grown under the same conditions, is unexpectedly uniform, amounting to .57 to .58 per cent citric acid." The fruits just referred to were of the same variety picked at the same time. The tests of acid content of incubated fruits were made later in the season, and these indicated a lower acidity than that of normally green or ripe fruits. At that time the requisite material was obtained from the Department of Horticulture, Cornell University.

During the past summer several varieties of tomatoes were grown in the Missouri Botanical Garden in order to furnish material for further pigment studies, and incidentally this material has enabled us to determine with greater care the acid content of tomato fruits, especially of different varieties, and likewise the comparative acidity of fruits direct from the field and of those of the same picking incubated for various intervals. The tests included below were made by pulping thoroughly a weighed quantity of the tissue (15 gm.), diluting with 150 cc. distilled water, employing for each titration 25 cc. of this solution diluted with distilled water to 50 cc., and titrating with n/10 NaOH, using phenolphthalein as indicator. Not less than two titrations were made in any case, and these were from one or more samples of tissue. The accompanying table

indicates the variety and condition of the fruit; quantities of n/10 NaOH required to neutralize; and the per cent of acidity in terms of citric acid.

TABLE SHOWING ACID CONTENT OF TOMATO FRUITS

Variety	Condition			Average no. of cc. of n/10 NaOH, to neutralise	Total per cent of acid as citric
	When picked	Interval or incubation	When titrated*		
Dwarf Stone	Ripe	0	Red	1.695	.52
Dwarf Stone	Half grown	0	Green	1.82	.56
Dwarf Stone	Half grown	Incub. 32° C. 10 days	Artif. yellow	2.135	.66
Dwarf Stone	Half grown	Lab. 24 days	Red	1.375	.42
Dwarf Stone	Half grown	Incub. 32° C. 10 days	Green	1.485	.46
Sparks' Earliana	Ripe	0	Red	1.695	.52
Sparks' Earliana	Half grown	0	Green	1.87	.58
Truckers' Favorite	Half grown	Incub. 32° C. 22 days	Artif. yellow	2.56	.79
Truckers' Favorite	Half grown	Lab. 24 days	Red	1.66	.51
Red Peach	Half grown	Incub. 32° C. 22 days	Artif. yellow	2.115	.65
Red Peach	Half grown	Lab. 24 days	Red	1.675	.52
Yellow Peach	Half grown	Incub. 32° C. 22 days	Artif. yellow	2.47	.76
Yellow Peach	Half grown	Lab. 24 days	Yellow	2.065	.64
Yellow Plum	Ripe	0	Yellow	2.12	.65
Yellow Plum	Half grown	0	Green	1.92	.59
Yellow Pear	Half grown	Incub. 32° C. 20 days	Artif. yellow	1.60	.49
Yellow Pear	Half grown	Lab. 24 days	Yellow	1.395	.43

* All fruits designated "red," "yellow," and "artificial yellow" were, at the same time, ripe.

The results above reported may not yet be as extensive as might be desired in order to follow closely the changes in acidity under different conditions; but they consistently point out certain relations of interest which may be briefly enumerated as follows: (1) A comparison of the acid content of green and normally ripened fruits was made, using Dwarf Stone, Sparks' Earliana, and Yellow Plum, all direct from the field. There were no marked differences between the green and ripe stages within the variety; yet the acidity of the green fruits of the red varieties in these tests is somewhat higher, while the acid content of the green fruits of the one yellow variety tested is somewhat lower. (2) Fruits of Dwarf Stone, Truckers' Favorite, Red Peach, Yellow Peach, and Yellow Pear which

were picked green and ripened in the incubator at 32–33°C. (10–22 days) exhibit a higher acid content than either those ripened on the vines or those ripened at the temperature of the laboratory. (3) There are considerable differences in the acidity of varieties, but judging from the results of these tests the normally ripened fruits of yellow varieties commonly contain as much acid as those of red varieties.

The several facts brought out by these tests render it obvious that there is now no sufficient evidence to justify relating pigmentation to total acidity. The acidity changes are, however, interesting in themselves, in these as well as in other fruits. No attempt was made to follow progressively any changes in acidity induced by conditions; but in titrating on one occasion, after an interval of two days, new samples of both red and yellow fruits which had been ripened in the laboratory, it was found that the acidity had noticeably declined since the previous titrations from the same lots of fruits.

We have reckoned the acidity of the tomato in terms of citric acid, as is customary. It should be noted, however, that while Bowman (3) and others report citric as the chief acid of the tomato, Albahary (1), on the contrary, gives .48 per cent as the malic acid content and .09 per cent as that of citric acid in the fresh fruits. The author last mentioned gives no indications respecting the variety or condition of the fruit employed. In a later contribution (2) he reports the results of analyzing tomato fruits in different stages of maturation, as follows: "1° le fruit vert avant l'apparition de la graine dans la pulpe; 2° le fruit vert au moment où la graine est complètement formée; 3° le fruit rouge arrivé à sa pleine maturation." In the second stage, corresponding to practically full grown, green, he finds .58, and in the ripe fruits .42 per cent of organic acids. This is in complete agreement with our findings. In the earliest stage of fruit development Albahary finds an acid content of only .116 per cent. Wehmer (5), after quoting Albahary (1) as to the percentage of the various acids in the fruit, remarks, "Die Acidität wechselt stark je nach dem Reifestadium (von 0,06–0,697% des Saftes auf Citronensäure berechnet)." He does not indicate the

source of these data, and certainly the smaller percentage given can refer only to the youngest stages of fruit development.

Graduate Laboratory, Missouri Botanical Garden.

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A METHOD FOR THE DIFFERENTIAL STAINING OF FUNGOUS AND HOST CELLS

R. E. VAUGHAN

Assistant in Plant Pathology, University of Wisconsin
Exchange Fellow in the Henry Shaw School of Botany of Washington University

In making histological studies of fungi on living or dead plant tissues the use of the stain known as "Pianeze IIIb" has been found very satisfactory in differentiating the fungus from the plant substratum, this differentiation occurring both in lignified and unlignified cell walls. The host tissue stains green and the mycelium a deep pink. This stain, devised by Dr. Pianeze for the study of cancer tissue,[1] is made up as follows:

Malachite green	0.50 gm.
Acid fuchsin	0.10 gm.
"Martius gelb"	0.01 gm.
Water, distilled	150.00 cc.
Alcohol, 95 per cent	50.00 cc.

Dr. Pianeze reports that it gives the following staining reactions: green in chromatin of resting or dividing nucleus, rose in cell protoplasm and membrane, and red in cancer bodies. For use with plant tissues the procedure is as follows: Wash in water or alcohol, stain in the undiluted mixture 15–45 minutes, remove excess stain in water, and decolorize in 95 per cent alcohol to which a few drops of hydrochloric acid have been added. For permanent mounts, clear with a carbol-turpentine mixture, remove clearer in xylol, and mount in balsam. Preparations of *Stereum*, *Corticium*, and *Polystictus* have been made with great success.

This stain is also valuable for staining germinated spores on the surface of a leaf. The procedure in this case is as follows: Infect marked portions of a leaf with a suspension of spores applied with a pipette, and place the plant under suitable conditions for fungous growth for 24–48 hours. Then permit

[1] Pianeze, G. Beitrag zur Histologie und Aetiologie des Carcinoms. Beiträge z. path. Anat. u. z. allg. Path. Supplement 1: 1–193. 1896. [cf. p. 58.]

the leaf to dry in the air, remove the area desired from the balance of the leaf, and place in a killing fluid. The best combined killing and tissue-clearing mixture for this purpose is one recommended by Dr. Duggar, composed of glacial acetic acid and 95 per cent alcohol. I have used equal parts of these agents most advantageously. This dissolves the chlorophyll, renders the leaf transparent or nearly so, and at the same time fixes the fungus with little plasmolysis. Allow the killing mixture to act for 24–36 hours; wash in 50 or 70 per cent alcohol, to remove the acid; and pass successively through the stain (15–30 minutes), water (2 minutes), acid alcohol (as short a time as possible), carbol-turpentine (until clear), xylol (until clearing agent is removed), and then mount in balsam. This process of differential staining has been successfully used with *Ascochyta Pisi* on pea, *Helminthosporium sativum* on barley, and *Phoma Brassicæ* on cabbage.

Pianeze's stain has not given as good results with the rusts as Durand's combination of Delafield's haematoxylin and eosin. Durand's stain[1] was not uniformly successful, however, and it was found that one of the chief difficulties often experienced finds its explanation in the killing solution which the stain follows. Flemming's solution, which was first used, gave very poor results. A modification of Gilson's mercuric chloride solution was found most satisfactory. This solution, as recommended by Dr. Durand, is made up as follows:

Water, distilled	60 cc.
Alcohol, 95 per cent	42 cc.
Acetic acid, glacial	18 cc.
Nitric acid, concentrated	2 cc.
Mercuric chloride, sat. aq. sol.	11 cc.

Diseased tissue may be fixed from 6 to 24 hours, then washed in 65 per cent alcohol, run through the alcohols, infiltrated with cedar oil, and imbedded in paraffin. This method is undesirable for nuclear structures, but gives excellent preparations for gross histological work.

Graduate Laboratory, Missouri Botanical Garden.

[1] Durand, E. J. The differential staining of intercellular mycelium. Phytopathology 1: 129-30. 1911.

TWO TRUNK DISEASES OF THE MESQUITE

HERMANN VON SCHRENK
Pathologist to the Missouri Botanical Garden

The diseases of the mesquite (*Prosopis glandulosa* Torr.) hitherto recorded are comparatively few in number; Heald and Wolf (5) enumerate seven from southern Texas as due to fungi. The pods are frequently affected by an anthracnose, *Glœosporium leguminum* (Cke.) Sacc.; the leaves are attacked by *Cercospora prosopidis* Heald and Wolf, a species of powdery mildew (*Erysiphe* ?), and by a rust, *Ravenelia arizonica* Ell. & Ev.; and a leaf blight due to some unknown cause is also mentioned. The large limbs and smaller branches show galls, evidently not due to insect attack, and the mistletoe (*Phoradendron flavescens* (Pursh) Nutt. is sometimes destructive. In addition to the above, the writer has frequently noted the weakening effect, particularly near the ends of branches, brought about by vigorous growths of the ball moss (*Tillandsia recurvata* L.). Birge (1) has given a good description of the effects of this plant on trees in Texas.

Of the insect injuries of the mesquite, that of the mesquite borer (*Cyllene antennatus* White) is of interest. The insect is described by Horn (6) as attacking mesquite wood in Arizona, but no description of its work is given. While I have not seen the insect at work in Texas, the holes found in the mesquite trees are so like those described for other species of *Cyllene*, notably *Cyllene robiniæ* Forster (10)—which attacks the locust—that the assumption seems warranted that the Texas insect is the one referred to by Horn. The tunnels extend straight through the bark into the heart-wood, and up and down in the latter, thus forming ideal channels for the entrance of fungous spores.

The only reference to trunk diseases which has been found is a brief statement by Havard (4), in an account of the mesquite, in which he mentions that "unfortunately it too often happens that the zones of the heart-wood are fissured, decayed or de-

tached from each other, so that it is difficult to get flawless boards."

In 1912 the writer found the older mesquite trees in the vicinity of San Antonio, Texas, seriously affected by a trunk disease, caused by one of the polyporous fungi. In one small field some twenty or more trees were found bearing the fruiting bodies of this fungus. Its distribution in the vicinity of San Antonio was general, and it is probable that it extends over a wider range, as evidenced by the finding of a sporophore by Underwood in the vicinity of Austin, in 1891.

Where the mesquite develops into a bush with several trunks, sometimes only one of the several trunks is affected, but in other cases several or all of them contract the disease. The age of the affected trees was difficult to estimate. The mesquite grows rather rapidly at first, but very slowly after eight or ten years. According to Sargent, trunks thirty years old may be seven to eight inches in diameter, while trees one foot in diameter are probably over one hundred years old. The trees found affected were from two to ten inches in diameter and all over twenty years of age, some of them probably very much older.

The decay is confined entirely to the heart-wood of the main trunks, extending from the ground up into the trunk for varying distances. The distribution is such that it is obvious that the fungus gains entrance through wounds in the trunk above the ground, chiefly through old branch stubs and borer holes, as is so frequently the case with trunk diseases of this kind. One instance was found which made it appear obvious that the holes made by the borer had served to give the fungus a start.

Sections of diseased trunks showed that the heart-wood was decayed to a greater or less degree (pl. 6 fig. 2). Mesquite wood has very sharply defined heart and sap-wood. The latter is light yellow or almost white and very narrow, being composed of but a few rings of wood, whereas the heart-wood is rich brown or reddish. The decay of the heart-wood begins near the center, and gradually spreads outward towards the bark; there is very little, if any, change in color (except that the decayed wood is a lighter shade of brown), and here and there irregular, thin lines of undecayed wood can be seen extending through the diseased

part. The decayed wood is very brittle, but still remains fibrous, that is, it does not crumble into powder like charcoal. It splits like sound wood, but is spongy and soft. The wood of the mesquite is very hard and heavy, a cubic foot weighing 47.69 pounds when absolutely dry. It consists of numerous, distinct medullary rays, and distinct but irregularly distributed bands of very thick-walled wood fibers, between which occurs a thinner-celled wood parenchyma. In the heart-wood the lumina of the cells of the latter tissue are usually completely filled with a yellow-brown substance, largely composed of tannin. McMurtree (8) found tannic acid in large quantities in mesquite wood, 6.21 per cent in the heart-wood, 0.5 per cent in the sap-wood, and 0.5 per cent in the bark. Besides tannin he found of materials other than tannin, insoluble in water but extracted by ether, 0.6 per cent in the heart-wood, 6.7 per cent in the sap-wood, and 1.84 per cent in the bark. A considerable number of large, open ducts are found in the early part of each wood ring. These also are filled with a yellow-brown substance similar to that found in the wood parenchyma.

The fine, colorless mycelium of the fungus spreads throughout the wood substance. Unlike *Polyporus rimosus* in locust wood (10), the fungus does not destroy the wood as a whole, but attacks only the heavily lignified groups of wood fibers. These are wholly destroyed, leaving holes or gaps between the vessels and wood parenchyma. The dissolution of the wood fibers evidently proceeds with great rapidity, starting with the secondary thickening of each cell. The cells disappear entirely, and in advanced stages of decay small masses of mycelium are the only evidence of their former presence. Although the wood parenchyma and the vessels are filled with hyphæ, they resist destruction almost completely,—a fact which may be connected with the very high tannin content of both of these tissues. The recent results of Wehmer (11), who found that for certain species of fungi tannin exerts a retarding influence on development, and the similar findings of Knudson (7), and of Cook and Taubenhaus (3), who state that "tannin has a tendency to retard or inhibit the growth of fungi," and that "the parasitic forms are more sensitive to the action of tannin than the saprophytic forms," lend support to this idea. Cook and Taubenhaus

also found that for the parasitic fungi tested, concentrations of from 0.1 per cent to 0.6 per cent were sufficient to retard growth. While the mere presence of considerable tannin may not entirely prevent the development of a fungus, it may retard its growth, and in the mesquite may explain the comparative immunity of the wood parenchyma to its attacks. The selective destruction of the wood fibers will serve to distinguish this form of decay from the other types of hardwood decay.

From the material found it was not possible to judge of the ultimate stages of the disease. In view of the fact, however, that sporophores four years old were observed, it seems that the resistance of a part of the wood structure is more or less permanent. No mesquite trees were found broken off as a result of the action of the fungus. It is conceivable, however, that very severe storms might break off trees weakened by the disease.

The fungus which causes the decay is *Polyporus texanus* (Murrill) Sacc. & Trott. The sporophores, which are annual and very distinct and easily recognized, develop around old knots. At the end of one year the sporophore dries and cracks (pl. 6 fig. 1, and pl. 7 figs. 1, 2), and many of them become badly eaten by insects. The latter may completely destroy the fruiting structure, thereby preventing the formation of new pilei from the original one. The sporophores occur either singly or in groups. In the latter case the oldest sporophore of the group is situated near the trunk, and gives rise during the second year to another pileus; from the latter a third one may grow out during the following year. This habit is well shown in pl. 6 fig. 1, and in pl. 7 fig. 1. The photograph reproduced in pl. 6 fig. 1 shows a group of three sporophores from below; the oldest one (in the back), dried and cracked; the second one formed immediately below the oldest one; and the youngest one developed at the side. This condition is also evident in pl. 7 figs. 1, 2. On the trees observed there was usually only one sporophore or a single group of sporophores, and while the internal decay extended in some cases for ten to twelve feet up and down in the trunk, in no case did the sporophores develop at more than one point.

Polyporus texanus (Murrill) Sacc. & Trott., was first described by Murrill (9) in 1904 from a specimen collected by Under-

wood on a mesquite (?) tree near Austin, Texas, in 1891. Murrill's description of this fungus is as follows:

"Pileus ungulate, attached by the vertex, 3 x 5 x 4 cm., surface fulvous to fuliginous, concentrically and radially rimose, especially in age, the separated areas imbricated; margin very obtuse, concolorous, context corky, concentrically banded, fulvous to umbrinous, very thin, only one-tenth the length of the tubes in thickness; tubes 3 cm. long, 2–3 to a mm., tawny chestnut, polygonal, edges thin, entire; spores ovoid, smooth, very dark brown, 1–2 guttulate, 8 x 10μ."

While this description was made from one specimen, the characterization is a good one and well defines the sporophores recently collected, and now in the herbarium of the Missouri Botanical Garden. One of the marked characters of the fruiting structure is the concentrically and radially rimose surface (pl. 7 figs. 1, 2) with imbricated areas, particularly in the older specimens. The tubes are very long, 2–3½ cm. (as stated by Murrill), and make up the larger part of the mass of the sporophore. The largest specimen found measured 9.5 cm. in width, 7 cm. in length, and 5 cm. in thickness. Using Ridgeway's color scale, the top is avellaneous gray, the tubes tawny, the substance antique brown (umbrinus of Saccardo's scale); near the margin the color is verona brown to warm sepia. Murrill's statement that the sporophore is attached by the vertex should be amplified, as many of the sporophores are practically dimidiate. With the additional material now available for study, the modified description of the fungus in question is as follows:

Polyporus texanus (Murrill) Sacc. & Trott. Syll. Fung. **21**: 272. 1912.

Inonotus texanus Murrill, Bull. Torr. Bot. Club **31**: 597. 1905.

Pileus ungulate, attached by the vertex or dimidiate, 4–9.5 cm. wide, 3–7 cm. long, and 4–5 cm. thick; surface avellaneous gray to fulvous, concentrically and radially rimose, especially in age, the separated areas imbricated; margin very obtuse, verona brown to warm sepia; context corky, concentrically banded, antique brown, very thin, only one-tenth the length of the tubes in thickness; tubes 2–3½ cm. long, 2–3 to a mm., tawny, polygonal, edges thin, entire; spores ovoid, smooth, very dark brown, 1–2 guttulate, 8 x 10 μ. Parasitic on living mesquite trees.

In the same locality in which *Polyporus texanus* occurred, one mesquite tree was found bearing a sporophore of *Fomes rimosus* Berk. This fungus causes the heart rot of *Robinia Pseudo-Acacia* (10), and it is of interest to note its occurrence on a new host. The specimen found is a typical sporophore of *Fomes rimosus*, measuring about two inches in length; unfortunately it was not recognized at the time of collection, and sections of the affected tree were therefore not made. In view of the destructive character of this fungus when found on *Robinia*, however, it is probable that it causes a similar heart rot of the mesquite. Further search will be made in the San Antonio region for additional evidences of its occurrence.

The wood of the mesquite is usually described as being very resistant to decay after it has been cut from the tree. For many years mesquite posts have been used in the southwest in preference to other kinds. Mesquite ties, foundation posts, etc., have also proved that the wood is very resistant to decay. This applies only to the heart-wood, however. The sap-wood is very short-lived, and where small trunks are cut, as is now frequently the case, and used for fence posts, the length of life is very short, —sometimes not over two to three years. The destruction of the sap-wood is due to a number of insects and saprophytic fungi, all of which are common on dead branches, posts, etc., in the vicinity of San Antonio. Of the more common fungi, the following were recently collected: *Polystictus Lindheimeri* B. & C., *Stereum Leveillianum* Fr., *Schizophyllum commune* Fr., *Lenzites protractus* Fr., and *Stereum albobadium* Schw.

The author acknowledges assistance from the following: Mr. Kearney Mason, of San Antonio, for permission to fell trees on his land and assistance in doing so; Dr. E. A. Burt, Mycologist and Librarian to the Missouri Botanical Garden, and Mr. C. G. Lloyd for aid in the identification of species of fungi.

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Explanation of Plate

PLATE 6

Disease of the mesquite due to *Polyporus texanus*

Fig. 1. View showing the manner in which a group of sporophores of *Polyporus texanus* grows on the trunk; also the lower surfaces of the sporophores.

Fig. 2. Two sections of diseased mesquite trunk showing the manner in which the wood is destroyed.

FIG. 1.

FIG. 2.

VON SCHRENK — TRUNK DISEASES OF MESQUITE

COCKAYNE, BOSTON.

Explanation of Plate

PLATE 7

Disease of the mesquite due to *Polyporus texanus*

Fig. 1. Side view of a group of sporophores of *Polyporus texanus* growing on a living mesquite tree.

Fig. 2. Front view of a group of sporophores of *Polyporus texanus* growing on a living mesquite tree.

FIG. 1.

FIG. 2.

VON SCHRENK — TRUNK DISEASES OF MESQUITE

COCKAYNE, BOSTON.

A TRUNK DISEASE OF THE LILAC

HERMANN VON SCHRENK

Pathologist to the Missouri Botanical Garden

A general discussion of diseases of the common lilac (*Syringa vulgaris* L.) was recently published by Klebahn (3). This author enumerates a number of diseases, such as the one of bacterial origin ascribed to *Pseudomonas Syringæ*, various leaf diseases due to species of *Microsphæra*, *Glæosporium*, and other leaf parasites, and a disease due to *Botrytis cinerea*. The major part of the work, however, deals with a disease due to *Heterosporium Syringæ* Oud., affecting the leaves, and a serious twig blight due to *Phytophthora Syringæ* Klebahn. Subsequent papers by various writers deal with one or the other of the diseases mentioned by Klebahn.

During recent years a destructive trunk disease of the common lilac (*Syringa vulgaris* L.) has been noted a number of times in the Missouri Botanical Garden, and in grounds in the vicinity of St. Louis. The affected plants were usually old bushes which had been more or less neglected, and the tops of the leading trunks were frequently dead. Long shoots from the root and others from the part of the trunks near the ground made a dense tangle around the main stem; on the latter sporophores of *Polyporus versicolor* were found in various stages of development, sometimes isolated, but more frequently in groups. Sections were made of the trunks on which this fungus was growing and it was found that such trunks were invariably diseased, while those close by, either from the same root system or from adjacent bushes—which were free from the fungus—were always sound.

In pl. 8 fig. 1 two affected trunks are shown cut at points about three feet from the ground. In both cases the larger part of the stem was alive, as evidenced by the presence of vigorous shoots along the entire length. Pl. 8 fig. 2, and pl. 9 figs. 1, 2 represent sections of lilac trunks taken from different bushes to show different stages of the disease.

The wood of the lilac is white in color, hard, and close-grained. In younger trunks there is no appreciable difference between heart-wood and sap-wood; as the trunks grow older, however, the heart-wood turns darker, and in those twelve years old, or thereabouts, it is distinctly darker than the rather thin, white sap-wood.

The disease first manifests itself in the inner heart-wood, frequently in close proximity to the holes made by the lilac borer. This lepidopterous insect (*Podosesia syringæ* Harris) (for whose identification I am indebted to Dr. E. P. Felt) has been found very destructive to lilac bushes, and, according to Beutenmüller (1), occurs from New England and the middle states westward to Colorado and southwest to Texas. Quoting from Beutenmüller's account: "The female deposits her eggs in patches on roughened or knotty places on the bark of ash and lilac. The eggs, according to Hulst, hatch in about six days, and the newly born larvæ at once eat their way through the bark into the solid wood. They run their channels longitudinally for about 8–10 inches through the wood. The larvæ pupate in slight cocoons after cutting their way to the bark, of which they leave only a thin outer skin. The pupation usually takes place early in May, and the moths emerge in about three weeks." Felt (2) briefly described the habits of the larva, stating that "a sign of its presence in midsummer being largely the sudden wilting of a shoot." He quotes from an observation made by Dr. Kellicott in which the latter states that he "watched 20 or more issue from a single tree in one day, and found that often there were more than one hundred in one tree." Felt recommends cutting and burning all infested wood in the early spring.

In the vicinity of St. Louis the lilac borer has been very active in recent years, judging from the fact that very few lilac bushes over five years old were found free from its attacks. Without much doubt the fungous spores get into the interior of the lilac trunks through the borer holes, and start to develop within the heart-wood on the edges of the borer holes. In pl. 9 fig. 1 two borer holes, still filled with pieces of the borings, can be seen in the lower right-hand trunk, and one small hole in this same section occurs in the sap-wood. The fungus, after it has begun to

grow in the hole, rapidly spreads up and down in the heartwood, and soon grows out from the center toward the bark. As the disease progresses, the wood is converted into a soft, pithy, white mass, having the consistency of corn-stalk pith. The line of demarcation between the sound and completely destroyed wood is very sharp (see pl. 9), resembling in this respect the type of decay caused by this same fungus in living catalpa trees ((5), pl. 26). The line between sound and decayed wood is so sharp that entirely decayed fibers adjoin perfectly sound ones. Between the wholly unaffected wood and the completely destroyed fibers, is a narrow ring of darker wood, which is, to all intents and purposes, sound; the wood cells are partially invaded by the mycelium of the fungus, and the lumina are filled with a yellow-brown liquid, which when seen in mass gives the section the dark color referred to. This liquid dries out in some places and leaves a brown amorphous substance, such as has frequently been found in the early stages of decomposition of hardwood wood fibers (6). It probably consists of decomposition products which are infiltrated into the sound wood immediately in advance of the fungus. In cases where the fungus starts in several centers, rings of the darker colored wood surround each decayed portion, a condition which is well shown in pl. 9 figs. 1, 2, where the fungus is growing in the center of the trunk and in addition in three more peripheral localities. In the lilac the brown substance referred to is ultimately destroyed (see the middle trunk of the lower tier, pl. 9 fig. 2, where the wood is destroyed up to the bark).

The completely decayed wood, which readily absorbs water, resembles pith, and in general is very similar to catalpa wood destroyed by *Polyporus versicolor* (5). It has some of the attributes of wood, i.e., it can be split, is fairly compact, and cannot be crumbled into powder. Sound lilac wood is very heavy and hard, and is composed almost wholly of very thick-walled wood cells, with small vessels scattered with considerable regularity throughout the annual ring; wood parenchyma is almost wholly absent. The hyphæ of *Polyporus versicolor* attack and very rapidly destroy the layers of secondary thickening of the wood cells. The middle lamellæ retain the nature of lignified fibers and resist destruction almost entirely, although

here and there some of them are dissolved, giving rise to small separated cell groups. Entire dissolution rarely takes place (this was also found to be true for diseased catalpa wood ((5) pl. 52)). With the removal of the secondary thickening, the resulting decayed wood has a skeletonized appearance. It has all of the elements, but these are very thin-walled. The fine medullary-ray cells are destroyed here and there, producing radial, isolated masses, but more frequently the decayed mass hangs together firmly. The dissolution of the layers of secondary thickening goes forward very evenly, bringing about the sharp dividing line between sound and decayed wood already referred to.

The only difference between the catalpa and lilac diseases is that in the catalpa the entire wood mass is skeletonized, whereas in the lilac hard areas of undestroyed wood fibers are left here and there, surrounded by decayed wood (pl. 9 figs. 1, 2). These masses are either entire rings (pl. 9 fig. 1) or irregular areas lying detached within the decayed parts, and represent portions of the heart-wood which for some reason have temporarily escaped total destruction; the wood fibers are filled with the yellow-brown substance, but do not otherwise differ from normal wood fibers. As the disease progresses, however, they are finally destroyed. This was made evident by the fact that in the upper parts of diseased trunks these immune areas were always found coexistent with the early stages of the disease, while lower down in the trunks, where the advanced stages of decay had been reached, they were practically absent. The temporary immunity may be due to the presence of more resistant groups of wood fibers, possibly also to a high concentration of decomposition products.

The development of the fungous mycelium from the center of the trunk out toward the bark differs radically from that of any other disease known to the writer. In most trees the destruction of wood by a fungus growing in the dead heart-wood is confined to the latter,—further growth ceasing as soon as the mycelium reaches the sap ring. As has been suggested by Münch (4), this is probably due to the fact that most mycelia of wood-destroying fungi require a balance between the amounts of oxygen and water contained in the wood fiber. Any undue

percentages of either may make the conditions unfavorable for further development.

In the lilac disease the fungus may grow outward concentrically in a regular manner (pl. 9 fig. 1). Very frequently, however, the fungus grows out into the sap ring at one side, at first slowly, then more rapidly. This is well shown in pl. 9 fig. 2, where four successive stages are represented by photographs. In the upper left-hand trunk the fungus has almost reached the bark, and in the three lower ones it has reached the bark and is gradually killing it. The probable explanation for this behavior is to be sought in the water content of the wood fibers. It was found that in many cases where the fungus actually grew up to the bark and through it, that on that side the lilac borer had been active; the wood fibers in the vicinity of the holes dried sufficiently to make growth possible for the mycelium, and as the destruction took place more drying occurred in adjacent areas until ultimately the whole sap region on that side was invaded and destroyed.

A number of water determinations were made of the wood fiber in the immediate vicinity of the growing mycelium, and the results compared with those obtained from normal sap-wood. In all cases the sap-wood about to be invaded was found to have a very much lower water content than the normal sap-wood. Unfortunately, it was impossible to get exact data which would indicate accurately the highest moisture content at which growth was possible; infected wood had obviously already reached and gone beyond that point, and as to sound new wood, even that which was near the borer holes, nothing could be postulated with certainty concerning its susceptibility or non-susceptibility to fungous attack. It would be an interesting problem to test the water susceptibility of *Polyporus versicolor* in its relation to lilac wood. It seems probable, however, that the drying out of one side of the trunks was at least one of the determining factors in the rather striking and exceptional method of growth of the fungus. Whether the fungus would eventually have destroyed the entire trunk it is impossible to state, because no such wholly destroyed trunks were found. There seems to be no reason, however, why this should not

occur; in fact, the right-hand trunk of the lower tier in pl. 9 fig. 2 has very little live wood left.

After the mycelium has reached the bark it grows through it, and fruiting bodies develop on the outside. The latter sometimes occur singly, but more commonly in linear groups parallel to the long axis of the trunk. Frequently one or more fruiting bodies grow out from the holes made by the borer. In the right-hand trunk in pl. 8 fig. 1 sporophores are shown growing out at the base of vigorous, live shoots; in the left-hand trunk the shoot is dead, having been killed during the year as the fungus invaded the wood from which the shoot was growing.

The sporophores found were typical of *Polyporus versicolor* L. This fungus is so common on the dead wood of various hardwoods that a detailed description is hardly necessary. It is interesting to note here that this is the second instance where this fungus attacks living plants. In the case of the catalpa the fungus grows vigorously only in the live tree; infected wood rarely, if ever, is decayed after it is cut from the tree. Many thousand posts of catalpa, the heart of which had been partially destroyed by *Polyporus versicolor*, have served as fence posts during the last ten years without showing a sign of decay of that part of the wood which was sound at the time of cutting. With the lilac it is different; the dead wood is just as subject to attack as is dead oak, beech, or gum wood.

The age at which lilac bushes are attacked has not been definitely determined. Those examined were about 15–20 years old (2½ inches in diameter). The trunk shown in pl. 8 fig. 2 was over thirty years old. It is probable that the disease is not serious until the bushes are ten or more years old, although this will depend somewhat on the rate of growth. Trunks 1½–2 inches in diameter were frequently found diseased. The effect of the disease is to gradually kill the top of the trunk; side shoots then develop farther down, which in turn are killed by the fungus, and eventually the trunk is broken off by the wind or snow.

The prevention of the disease is possible by continued attention to the borers. A careful examination for the latter should be made in June or July, and if any are present these should be killed by means of a wire, and the holes—after antiseptic treatment with some coal-tar compound—plugged. Painting

or brushing the lower parts of trunks with whale oil soap, or its equivalent, should also prove of value. Wherever a diseased trunk is found it should at once be cut out and burned. All dead wood in the neighborhood of lilac bushes should be cleaned up, so that the chances for infection may be reduced.

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Explanation of Plate

PLATE 8

Trunk disease of lilac due to *Polyporus versicolor*

Fig. 1. View of two diseased lilac trunks showing the sporophores of *Polyporus versicolor*, and the manner in which living branches grow from diseased trunks.

Fig. 2. Sections of an old diseased lilac trunk showing the decayed heart-wood.

FIG. 1

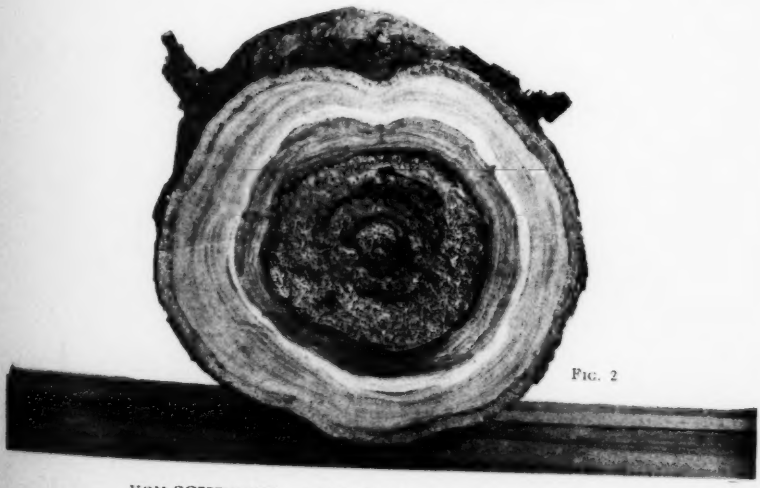

FIG. 2

VON SCHRENK—TRUNK DISEASE OF LILAC

COCKAYNE, BOSTON.

Explanation of Plate

PLATE 9

Trunk disease of the lilac due to *Polyporus versicolor*

Fig. 1. Sections of three diseased trunks showing early stages of the disease.

Fig. 2. Sections showing progressive stages of the lilac trunk disease.

Fig. 1.

Fig. 2.
von Schrenk — Trunk Disease of Lilac

Cockayne, Boston.